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FEMALE MATE CHOICE IN A MAINLAND POPULATION OF THE STRAWBERRY POISON FROG, OOPHAGA PUMILIO

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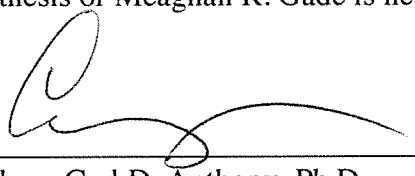

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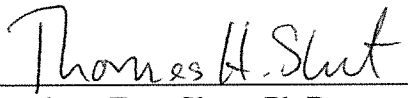

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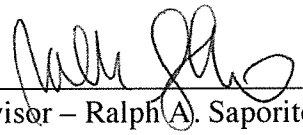

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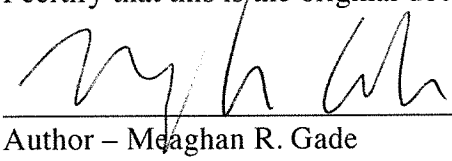

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Chapter 2

AN EXPERIMENTAL TEST OF FEMALE MATE CHOICE WITHIN A MAINLAND POPULATION OF *OOPHAGA PUMILIO*

ABSTRACT

Sexual selection and female mate choice are driving forces in the evolution and maintenance of biological diversity, and may function in the evolution of many secondary sexual characteristics throughout multiple taxa. Female mate selection has been experimentally shown to drive the evolution of color between the polytypic populations of the dendrobatid frog, *Oophaga pumilio*, in the Bocas del Toro archipelago in Panama. However, limited studies exist examining biologically relevant female mate choices within a single population. Using analogous experimental arenas from previous studies, females were presented with two males exhibiting phenotypic extremes from within the same population at La Selva Biological Research Station in Northeastern Costa Rica. Females did not prefer males with certain color phenotypes, however they did tend to choose the larger male or the first male encountered. Size may indicate a male's ability to defend reproductive resources, and close proximity of a male may be important in energy optimization for the females. The present results differ from those in Bocas del Toro whereby females chose males with specific color phenotypes, possibly because in the present study with realistic mating situations, the level of color variation is not great enough to infer fitness advantages of the male. Further, measuring female choice by time in proximity in an experimental arena may be inadequate due to its artificiality, especially considering the complex courtship exhibited in this species which is difficult to replicate in such lab experiments. Future studies investigating naturally occurring mating pairs are

needed to further understand the specific parameters females use to assess potential mates.

INTRODUCTION

Sexual selection has been well documented throughout the animal kingdom as having important ecological and evolutionary consequences. The two main forms of sexual selection are male-male competition and female mate choice. In male-male competition, intraspecific males compete with each other for direct access to females or essential resources to attract females (Gould and Gould, 1989; Berglund et al., 1996; Crothers et al., 2011). Since females tend to be the limiting resource to male reproductive success, males strongly compete for access to females, facilitating the evolution of secondary sexual traits (Berglund et al., 1996). The second form of sexual selection, female mate choice, whereby females directly choose a mate based on certain characteristics, can place large selective pressures on certain male traits, and is a fundamental force in both driving diversification and maintaining biological diversity (Trivers, 1972; West-Eberhard, 1983; Andersson, 1994; Maan and Cummings, 2009).

Females of many species tend to be the more discriminatory sex because they invest greater amounts of energy into different aspects of mating, such as gamete production, mate sampling, and parental care in the form of feeding or defending offspring (Trivers, 1972; Guevara-Fiore et al., 2010). Due to the increased energy investment, it is crucial that females choose a mate who has the potential to provide material benefits, such as food, protected territory, parental care, and genes that will result in higher offspring fitness (Andersson, 1994). Female mate choice is commonly utilized by multiple taxa (e.g., birds, insects, fish, lizards; Atatalo et al., 1984; Oneal et al., 2007; Noonan, 1983; Censky, 1997), and typically occurs by way of females

assessing male quality using information contained in specific phenotypic traits (i.e., male secondary sexual characteristics).

Female mating preference has been demonstrated to function as a potential mechanism for the evolution of various secondary sexual characteristics in males. For example, the elaborate plumages of male house finches (*Carpodacus mexicanus*) can influence a female's choice, whereby females tend to choose males with brighter plumage coloration. Color in male finches is a reliable, condition-dependent trait used by females to assess the relative foraging success of a male, and his ability to provide food (Hill, 1990; Hill, 1991; Hill and Montgomerie, 1994). In the lizards *Eumeces laticeps* (Cooper and Vitt, 1993) and *Ameiva plei* (Censky, 1997), females tend to choose larger males. Body size may serve as an indicator of strength and ability to protect a female, or age and consequently, mating experience and survivorship ability (Censky, 1997). Further, females of certain amphibian species have been observed to choose mates based on a variety of male signals. Many female anurans utilize the structure of advertisement calls to determine the quality of a male (Ryan and Rand, 1990; Pröhl, 2003), with more complex calls requiring greater energy and suggesting greater fitness. Additionally, some salamander and frog species use color signals as an indication of the value of a male by inferring territorial aggression, diet quality, or predator avoidance abilities (Summers et al., 1999; Anthony et al., 2008; Maan and Cummings, 2008; Acord et al., 2013).

Secondary sexual traits are important to the reproductive success of many species, and systems in which conspicuous sexual displays are used provide a particularly good opportunity to gain a better understanding of sexual selection. Anurans, especially poison frogs in the dendrobatid family, are an excellent system for these types of investigations

because they exhibit female mate choice and have conspicuous coloration, which factors into sexual selection (Summers et al., 1999; Maan and Cummings, 2008). The salient coloration of these frogs is thought to have evolved cooperatively via natural selection as an aposematic signal to warn predators of the alkaloid-based defenses in their skin (Darst et al., 2006; Saporito et al., 2007a), and via sexual selection as an indicator trait to influence female mate choice (Summers et al., 1999; Maan and Cummings, 2008). The strawberry poison frog, *Oophaga pumilio*, has become a model dendrobatid species to study the significance of coloration on female mate choice due to its vivid coloration and strong female parental care, which includes female egg transport and daily tadpole feeding, but with limited male care (Trivers, 1972; Savage, 2002; Siddiqi et al., 2004). Amplexus does not occur in this species, therefore, males cannot force mating, subsequently allowing females to have free choice of mates (Meuche et al., 2013). While male advertisement calls have been shown to be an important factor in female mate choice (Pröhl, 2003), it is possible that females are also influenced by the coloration of males. This is especially evident in populations of *O. pumilio* in the Bocas del Toro archipelago off the northeastern coast of Panama. Populations in this archipelago exhibit extreme color polytypism with over 15 different color morphs, ranging across the color spectrum (Summers et al., 2003; Maan and Cummings, 2008). This extreme radiation of color has been hypothesized to be a result of sexual selection (Maan and Cummings 2008; Maan and Cummings, 2009; Hegna et al., 2011; Crothers and Cummings, 2013).

Multiple lab-based experimental studies on these polytypic populations have investigated the evolution and maintenance of color diversity between these populations. In general, these studies have presented a female of a particular color morph with a

choice of two males: one of the same color morph from the same island (native) and one from a different island and color morph (naïve; Summers et al., 1999; Maan and Cummings, 2008). These experiments suggest that females tend to choose "native" males with similar coloration and patterning, indicating that females use both color and pattern of males as a visual cue in choosing a mate (Graham-Reynolds and Fitzpatrick, 2007). While these studies have provided a mechanism for the evolution of extreme color polytypism in Bocas del Toro, the manner in which females choose mates in their natural environment is not well understood. In a natural setting, females do not have the opportunity to choose between native and naïve males, because these populations and color morphs do not occur sympatrically (i.e., in most cases different colored frogs occur on different islands). While females may "prefer" to assortatively mate with their same color morph when given contrasting options, in nature, they must "choose" males based only on what is biologically available (Richards-Zawacki et al., 2012). While understanding the choice preferences in this species is important, very few studies have identified how females choose mates under a biologically relevant scenario.

Interestingly, females become less choosy when presented with males that have similar dorsal coloration to each other and to that of the female (Maan and Cummings, 2008). This limited variation in dorsal coloration is probably a more accurate reflection of the variation found within a population of *O. pumilio.* According to Maan and Cummings (2008), it seems females are either incapable of discriminating between similar morphs, or are impartial towards males with very similar dorsal coloration to their own. This is particularly important when considering sexual selection within populations of *O. pumilio*, because these individuals have limited color variation. While the majority of

research on sexual selection has been conducted between populations in Bocas del Toro in northwestern Panama (Summers et al., 1999; Graham-Reynolds, 2007; Maan and Cummings, 2008; Maan and Cummings, 2009; Crothers, 2011; Richards-Zawacki et al. 2012), the geographic range of *O. pumilio* lies mainly in the Caribbean lowlands of southern Nicaragua through Costa Rica (Savage, 2002). Due to the limited number of studies conducted on mainland populations, and the lack of evidence of biologically relevant mate choice tactics, it is not entirely clear how females choose mates within their own populations.

Recently, field studies on mate selection in mainland populations of *O. pumilio* in Costa Rica have found that females tend to choose the calling male who is closest in proximity to a female regardless of his acoustic quality, body condition, or territory size (Meuche et al., 2013). Meuche et al. (2013) suggested that female *O. pumilio* utilize a simple mate sampling strategy whereby females base their decision on closeness of a male in order to reduce searching costs associated with finding a mate. However, this study did not examine coloration or pattern of males, which could be used by females in mate selection. Dreher and Pröhl (2014) examined both male brightness and auditory stimuli in mate selection on the mainland in Costa Rica, and found that females are capable of using both of these signals when assessing potential mates; however, auditory signals appear to be the more important cue, especially with respect to detecting and assessing males from a distance. It has been suggested that color signals are used for close range assessment (Dreher and Pröhl, 2014), although this has not yet been tested. Research is needed to investigate the specific role of male color and pattern in mate selection within mainland populations of *O. pumilio*.

The goals of the present study are to examine how male color (hue and brightness), pattern, and body size (SVL and mass) influence female mate choice in a mainland population of *O. pumilio* from Costa Rica in an experimental setting. By presenting females with males with whom they would realistically have the opportunity to mate with in nature, the choice tactics of females can be assessed directly. Additionally, a comparison of female mate selection can be made between a mainland population in Costa Rica and those in Bocas del Toro by using similar methods from previous studies on *O. pumilio* in this archipelago (Summers et al., 1999; Maan and Cummings, 2008; Maan and Cummings, 2009). On the basis of previous studies, it is hypothesized that male dorsal phenotype (hue, brightness, and pattern) will influence female mate choice.

METHODS

Study Site - This study was conducted at the Organization for Tropical Studies, La Selva Biological Research Station in northeastern Costa Rica (10°26' N, 83°59' W) from 28 May 2014 to 25 June 2014. Three locations were used for frog collection at La Selva: Huertos Plot (H), Successional Plot (SP), and Sendero Tres Rios 3950-4150 (STR; FIG. 1). The Huertos plot is an abandoned tropical hardwood plantation (*Hyeronima alchorneoides)* with rows of large trees and nearly complete canopy cover (Stynoski, 2009; Traub, 2014). The successional plots are a series of 100m x 50m plots that are managed and maintained at certain time periods (0-1 year, 1-2 years, 3-4 years, 4-5 years) for study of forest succession. Plots during all stages of succession were used for this study. The STR plot, located between trail markers STR 3950-4150, consisted of an open

swamp area surrounded by secondary forest with canopy cover and understory plants (Traub, 2014). These locations were chosen due to high frog density and previous work that showed variation in color (hue), brightness, and pattern in *O. pumilio* at each location (Traub, 2014).

Pilot Study - A pilot study was conducted to determine the available variation in male hue and brightness at each site, and to determine the "extremes" of each color trait at each location. Extremes of each color trait were determined to ensure collection of the full range of each trait in the experimental arena study (described below). To do this, 20 male frogs were collected at each location (H, SP, and STR) and brought back to a lab at La Selva, where a spectrophotometer (details below) was used to measure hue and brightness of individual frogs. Color metrics were quantified using the JAVA-based program CLR (version 1.05, Montgomerie, 2008). Histograms were constructed to visualize the total range of variation in color and brightness at each location, and the upper and lower third of the range was used to determine the extreme limits of each trait. These "extreme" values were used in selecting males to be included in the experimental study. Pattern was not quantified in the pilot study, but still included in the "extreme" male selection by an observer (Meaghan Gade or Michelle Hill) estimating the dorsal patterning as either "patterned" or "not patterned".

Frog Collections and Measurements - Males were collected with a specific set of traits (orange/red, dull/bright, no pattern/pattern) to represent all the possible combinations of each trait extreme. Eight males were used to represent each of the possible combinations of color and pattern traits (Table 1). Using this specific set of eight males, an equal sampling of each individual trait, and combination of traits was used in

the experiment. A total of seven groups of eight males were collected, and each group of males was used in 28 of the 190 trials (see details below). Selection of extreme males were based only on the combination of hue, brightness, and pattern, due to logistic and factorial limitations; however, both color metrics and size (mass and SVL) were included in analyses.

On each male collection day, at least 10 males were captured at each site. Males were distinguished from females by their vocalizations and the presence of a pigmented gular sac (Bunnell, 1973). For each male, the hue and brightness was measured using an Ocean Optics (Dunedin, FL) USB 4000 UV-VIS spectrophotometer, with a PX-2 pulsed xenon light source and a R400-7-SR reflectance probe with a 400 μm core diameter. The spectrophotometer was held in a fixed 90° position and approximately 6 mm from the dorsal surface of the frogs. White standard measurements were taken between each individual frog using a Labsphere certified reflectance standard. Three random points along the dorsum of each frog were selected and three readings were measured at each location. An average of the three readings was calculated for use in the analysis. Hue and brightness were calculated similar to the pilot study using the Java-based program CLR (version 1.05, R. Montgomerie 2008), following the equations detailed by Endler (1990). The eight males that fit all the extreme combinations were kept in terrariums at ambient temperature for no more than six days prior to being used in an experiment. Leaf litter and associated prey in the terrariums was replaced daily. The remaining males not used in the experiment were returned to the field within 24 hours.

Dorsal patterning was also quantified for each male by photographing the dorsum of each frog using a Sony Cyber-shot point-and-shoot camera attached to a tripod 15 cm

above each frog. Each photo was analyzed using the computer program ImageJ (version 1.48, Rasband, 2014). The percent of the dorsum covered with pattern was calculated by dividing the total area of the dorsum by the sum of the area of each spot on the dorsum. Snout-to-vent length (SVL) was measured to the nearest 0.01 mm using digital calipers, and only adult individuals, ranging from 19-24 mm were used in the trails (Savage, 2002). Mass was measured using a Pesola PPS200 digital pocket scale to the nearest 0.01 g.

Females were collected from each of the three sites (H: n=72; SP: n=70; STR: n=66) on the morning they were used in a trial. The females were allowed to acclimatize to the lab in large Ziploc® bags filled with leaf litter for at least two hours before being used in a trial. Hue, brightness, pattern, SVL, and mass of each female were measured using the same methods as described above. All females were measured after being used in an experimental trial, to avoid adding potential stressors and altering their behaviors and possibly mate choice.

Laboratory Mate Choice Experimental Set-up - To assess female preference for certain phenotypic traits of males, a series of female choice experiments in a three-chambered experimental arena, modified from previous mate choice studies, were conducted (see Maan and Cummings, 2008; Maan and Cummings, 2009; Crothers et al., 2011). The chambers consisted of two 15.2 x 15.2 x 15.2 cm UV-transparent clear acrylic "male" boxes and one 30.5 x 15.2 x 15.2 cm UV-transparent clear acrylic "female" box (FIG. 2). The visual background and floor of each male chamber was black, to increase contrast of the males to their background. The right and left walls of the female chamber were blacked out to reduce any outside distractions, leaving the front wall free to observe

the male chambers, the back wall open to the experimental observers, and the ceiling open for light. The floor of the female chamber was black with a grid drawn on with silver Sharpie ® (FIG. 3). The grid consisted of 12 quadrants that were used to track female movement during the trials. The 4 quadrants closest to the male chambers were each 4 cm x 7.5 cm and the 8 remaining quadrants were each 5.5 cm x 7.5 cm. The 4 cm quadrants were specifically chosen for direct comparisons to previous experiments (Maan and Cummings, 2008; Maan and Cummings 2009). Experiments were carried out in a light controlled room that mimicked the lighting in the forest by using one 22-inch UV 20W bulb and two 50W "daylight blue" incandescent bulbs filtered by two green-blue filters (Lee 728, CyanGel 4315) suspended over the male chambers (Crothers et al., 2011).

Males were placed into one of the two chambers following a randomly assigned schedule of male matchups. Males belonged to a group (A-G) and each had a specific number (1-8) depending on which extreme trait combination it possessed. Each male was used in eight different trials: 4 trials within a group and 4 trials between groups. Males were placed on either side of the chamber 4 times to reduce side bias, used a maximum of 2 times per day, and were never used in successive trials.

Male *O. pumilio* exhibit aggressive and territorial behavior with other males, and use vocalizations and occasionally physical combat to protect their territory (Bunnell, 1973; Pröhl, 2005). To reduce any potential that males behaved differently when observing another male, which could affect female choice, males were shielded from seeing each other using a visual barrier (Maan and Cummings, 2008; Maan and Cummings, 2009). Additionally, to suppress any calls that males made in the chambers, a

speaker, placed 10 cm behind the male chambers, broadcasted pre-recorded cicada calls, which are a common sound in the forest at La Selva, and have been shown to provide acoustic interference with *O. pumilio* (Paez et al*.,* 1993; Wong et al., 2009).

Once the males were placed in the chambers, a randomly selected female was placed in the female chamber and allowed to acclimatize for 5 minutes. A different female was used for each trial. During this acclimatization period, the female's view of the males was blocked using black cardboard as a visual barrier. Following this period, the visual barrier was removed and an observer (Meaghan Gade or Michelle Hill) using the computer program Event P.C. recorded female behavior and movement. The following behaviors were recorded for 15 minutes: (1) time each female spent in each quadrant, (2) time each female spent on each wall, (3) the number of times she looked at either male, and (4) the number of times she entered the side of the arena of either male (hereafter referred to as intervals). After each trial, the chambers were wiped clean with a 10% methanol solution to remove any potential chemical cues between trials. Each trial was video recorded using a Sony Cyber-Shot point-and-shoot camera.

Statistical Analysis- The Bradley-Terry model (BTm) was used to determine the male characteristics that were most attractive to females when choosing a mate (Bradley and Terry, 1952). The model was used to determine the "winning" male in each trial based on the time spent, number of looks, or number of intervals the female spent with each male. The BTm allows the relative importance of multiple independent variables (i.e., hue, brightness, pattern, SVL, and mass) to be measured simultaneously (Stuart-Fox et al., 2006; Turner and Firth, 2012) and accounts for males in multiple contests facing different males, but not all males encountering every other male (Stuart-Fox et al., 2006).

The latter point is important to the present experimental design, because each male did not compete against every male used in the study.

Female choice was estimated using a series of different analyses. The most conservative and best predictor of female choice was analyzing the total time a female spent in the quadrant closest to the male chambers (L1L2 and R1R2; see FIG. 3). This is consistent with the analyses of other similar studies (Maan and Cummings, 2008; Maan and Cummings, 2009; Crothers et al., 2011). Additional analyses included the amount of time a female spent in all quadrants of each side (L1-L6 and R1-R6; see FIG. 3), the total number of times a female looked at each male, and the number of times she entered the side of each male chamber (intervals). In all of these analyses, the male scored as the "winner" was based on which one received more attention (time, looks, or interval number) from a female. The "status" of a male (winner or loser), the percentage of the degree of winning versus the loser, and the characteristics of each male (hue, brightness, pattern, SVL, and mass) were used to generate the predictions of the model using the BTm function in the BradleyTerry2 package in R (R Core Team, 2013).

The BTm was also used to determine if a "home field advantage" existed for male choice. This was analyzed by establishing the side the female started on when the visual barrier was removed, following the female acclimation period, and then determining whether that male was most likely to be the ultimate winner of the trial.

RESULTS

Pilot Study - In order to determine the range of variation in male color available for female selection, 20 male frogs from each of the three locations within La Selva were

collected and their hue and brightness were characterized. The average values and ranges for hue and brightness for each location are in Table 2. Frequency histograms for hue and brightness were constructed by combining data from each of the three different locations (data not shown). The upper and lower third of these distributions were chosen as the "extreme" values for male hue and brightness, and males that were found to possess these extreme phenotypes were those presented to females in the experiment. Males were categorized as "red" if their hue was < 0.42, and "orange" if their hue was > 0.47. Males were categorized as "dull" (the lower extreme of brightness) if their total reflectance flux was < 66, and bright (the upper extreme of brightness) if their total reflectance flux was > 86.

Male Traits - The average reflectance curves for each numbered (1-8) male across all seven groups (A-G) are presented in FIG. 4. These reflectance curves represent the average hue and brightness for the dorsum of each of the frogs. The color metric values for these males and all other males collected and measured to find the specific males used in the present study are in Appendix 1.

Bradley Terry Model (BTm) - A total of 190 trials were conducted in which females were offered a choice between males with the extreme phenotypic combinations, and female choice was analyzed in 5 different ways (L1L2, R1R2, all quadrants, number of looks, number of intervals, home field advantage) using the Bradley-Terry model.

L1L2, R1R2 – Male mass was a significant predictor of female mate choice (P = 0.017) when analyzing the total time females spent in the four quadrants closest to the male chambers, and females associated more strongly with males that had larger masses (Table 3). Female preference was not influenced by hue, brightness, pattern, or SVL of a

male (Table 3) There was no significant interaction between any of the traits in this analysis (data not shown).

All Quadrants (L1-L6, R1-R6) – Male mass was a significant predictor of female mate choice ($P = 0.005$; Table 3) when analyzing the total time females spent in all the quadrants on either side of the male chamber, whereby females associated more strongly with larger males. Hue, brightness, pattern, and SVL of males were not significant predictors of female choice (Table 3). There was no significant interaction between any of the traits in this analysis (data not shown).

Number of Intervals and Looks – Male mass was a significant predictor of female choice ($P = 0.012$) when considering the number of times females entered either side of the arena for all quadrants (i.e., entered L2, L4, L5 or R1, R3, or R6; refer to FIG. 3). Females tended to choose males with larger masses. Hue, brightness, pattern, mass, and SVL were not significant predictors of female choice when considering the number of times females looked at either male (Table 3). There was no significant interaction between any of the traits in this analysis (data not shown).

Home Field Advantage - Males on the initial side females started on were a significant predictor of which male would be chosen (*z-score* = 3.76, $P < 0.001$, odds ratio = 2.2). The odds in favor of being chosen for "home" males were twice as great than "away" males.

DISCUSSION

Coloration of male individuals has been shown to drive female mate choice between polytypic populations of *O. pumilio* in the Bocas del Toro archipelago in northwestern

Panama (Summers et al., 1999; Graham-Reynolds and Fitzpatrick, 2007; Maan and Cummings, 2008). However, in the present study, which examined female mate choice within a population of *O. pumilio*, no evidence was found to support the hypothesis that male coloration or patterning influences female mate choice. Instead, it seems as though mass and proximity of males to females most strongly influence the way in which females are choosing mates. Further, there is some evidence that females use different male signals depending on which ones are immediately available to them.

Size is a common characteristic used by females when selecting mates in many different organisms, such as reptiles (Cooper and Vitt, 1993; Censky, 1997; Weatherhead et al., 2002), toads (Wilbur et al., 1978; Boell and Linsenmair, 1998), mammals (Pack et al., 2012), arthropods (Harari et al., 1999; Hoefler, 2007), fishes (Thunken et al., 2012; Conte and Schluter, 2013), and birds (Price, 1984; Jawor et al., 2003; Christensen and Kleindorfer, 2007). Large body size may indicate a male's ability to procure resources, such as oviposition sites and food (Searcy, 1979), fecundity (i.e., large body size is related to greater fecundity; Fairbairn, 1988), or survivability (Censky, 1997). However, in *O. pumilio*, male size is not likely indicative of fertilization success because female clutch size is small (3-5 eggs) and amplexus does not occur (Savage, 2002; Pröhl, 2003). Instead, size may indicate hatching success of fertilized offspring. Males of this species exhibit limited parental care, in which they defend fertilized clutches within their territories, and will deposit bladder water on them daily to keep them moist until hatching (Pröhl and Hödl, 1999; Summers et al., 2003). If larger males are better at protecting their territories, this may also allow them to better defend clutches, which may lead to a greater chance for offspring success. Defense of food resources within a territory may

also be important (Staudt et al., 2010), because these frogs obtain their alkaloid chemical defenses from their diet of mites and ants (Saporito et al., 2007b). Therefore, if a male is more capable of defending a territory that has greater food resources, and subsequently obtains greater alkaloid defense, he will be better protected from predators. Territory defense is also important because males may be defending reproductive resources such as tadpole rearing sites (e.g., bromeliads), which are important for females (Donnelly, 1989). Larger males may be more capable of defending territories that have the best sites for females to rear tadpoles. Females may be assessing mates based on their size to determine the quality and quantity of potential offspring rearing sites, which could lead to greater offspring fitness.

Females were approximately two times more likely to select males that they first encountered in the present study. This is important when considering mate search strategies, because a female's ultimate choice is determined by the amount of effort placed into searching for a mate, and the tradeoffs associated with that search, such as more time exposed to predators or time lost to foraging (Choudhury and Black, 1993; Johnson and Basolo, 2003; Meuche et al., 2013). Female choosiness often increases when it is beneficial and the costs do not outweigh the benefits (Fawcett and Johnstone, 2003; Meuche et al., 2013). In mainland populations of *O. pumilio*, the mate sampling tactic has been described as one in which females select the closest calling male regardless of the physical traits a male possesses (Meuche et al., 2013). Therefore, color traits within mainland populations of this species may not signal any reproductive or fitness advantages. Consequently, investing a great deal of energy into searching for a male with certain characteristics may not be beneficial to females.

Acoustic signaling is commonly used by male anurans of multiple species in courting females, such as tungara frogs (Ryan et al., 1982; Ryan and Rand, 1990), spring peepers, (Forester and Czarnowsky, 1985), natterjack toads (Arak, 1988), and certain microhylid frogs (Hoskin, 2004). In these species, females use certain parameters of a male's call such as frequency, amplitude, or pitch, to assess the quality of a male. In *O. pumilio*, total calling activity of a male has the largest influence on male mating success (Pröhl, 2003). However, calls from a male *O. pumilio* may only serve to announce his presence, and guide a female in his direction. In this scenario, the amount of calling from a male may still increase his fitness, because otherwise, a female would be incapable of finding a male in the dense secondary forest *O. pumilio* prefers to inhabit (Guariguata et al., 1997; Savage, 2002; Pröhl, 2003). Therefore, in the present experiment, females may have used the next available and informative signal, such as mass or proximity, since males were visible and did not need to advertise their location. Mass of a male was the best predictor of female choice, and females tended to choose the largest males. However, females were also more likely to choose males that they first encountered, which may reduce the energy costs associated with searching out another mate. Therefore, females of this species may be capable of using multiple cues depending upon what is immediately available when assessing and selecting mates. This species' ability to use multiple cues when choosing a mate requires further investigation.

While no evidence was found to support the hypothesis that female selection is driven by coloration, it is possible that coloration on the mainland is instead more important in male-male interactions. Studies with the Bocas del Toro archipelago populations of *O. pumilio* have found that dorsal brightness influences certain male-male

behavioral interactions, such as territorial disputes (Crothers et al., 2011). Brighter males tend to be more aggressive and more capable of defending their territories when compared to duller males (Crothers et al., 2011; Crothers and Cummings, 2015), but this has yet to be studied in mainland populations of *O. pumilio*. It is possible that brightness in the La Selva population of *O. pumilio* may play a role in predicting such male-male interactions. Certain aspects of the phenotype of these frogs, including color, may have evolved via both natural selection (predation pressure) and sexual selection (male-male interactions), whereas other attributes of males, such as body size and calling behavior, have evolved primarily through female mate choice (Graham-Reynolds and Fitzpatrick, 2007; Maan and Cummings, 2008; Brown et al., 2010; Crothers and Cummings, 2013).

The studies in the Bocas del Toro archipelago investigated mate choices between different populations, and suggested that females assortatively mate. However, results from trials in which males from different populations with similar phenotypes were presented to a female demonstrated that females do not show any preference between the males (Maan and Cummings, 2008). This scenario may more accurately characterize the level of variation in males within a population, and therefore, is more likely representative of realistic mating decisions. The current study investigated female mate choice within a population, and found that females do not seem to use male coloration when selecting a mate in an experimental setting. In natural mating situations in *O. pumilio,* females may not use male color or other traits to assess the quality of a mate. However, it is also possible that the lack of evidence for male color phenotype impacting female choice in the current study is the result of the experimental design. The males that were selected to present to the females had the most "extreme" color combinations

available at La Selva. Only the absolute brightest and dullest, reddest and most orange, and most and least patterned males were chosen for the trials. This may have been an unrealistic representation of the males that females actually come into contact with in nature. These "extreme" males are rare forms in the population, and may not be commonly seen by females. Furthermore, previous studies have suggested that females tend to choose males that look most similar to themselves (i.e., assortative mating; Graham-Reynolds and Fitzpatrick, 2007; Maan and Cummings, 2008). Unfortunately, the specific traits of the females were not measured in the present study, and therefore, it is not known if this is true of females at La Selva. Further studies examining both male and female color metrics will likely shed light on the possibility for assortative mating. It is also possible that using proximity and time spent with a male by a female as indirect evidence for female preference is not a good measure of female choice. Previous studies using repeatability tests of female choice on different fish species have revealed discordance between time association and female choice (Gabor, 1999; Aspbury and Basolo, 2002; Egger et al., 2008). It is possible that the design in the present study was not suitable for predicting female choice. Additionally, considering courtship in this species can last anywhere from 15 minutes to over an hour (Savage, 2002), a 15 minute association time may not have been an appropriate amount of time to judge female choice. Further, courtship in *O. pumilio* is composed of a series of complex behavioral interactions (Limerick, 1980; Savage, 2002) that were not and could not be replicated in these experimental trials.

Conclusions- Females seem to be influenced by mass and the proximity of a male when selecting between two potential mates within their own population. Mass may

indirectly signal fitness to a female due to a male's greater ability to defend food, reproductive resources, and egg clutches found within a males territory. Proximity of a male to a female may be important because of the need of females to optimize their energy when searching for potential mates. Coloration may not signal any direct fitness advantages to a female, and therefore, it may not be worth the female's energy to search out males with a specific trait or phenotype. Coloration may instead be involved in predator avoidance or male-to-male interactions. Future investigations of naturally occurring mating pairs and their color metrics may be useful in distinguishing the specific parameters females use to choose mates in a natural setting.

LITERATURE CITED

Acord, M.A., Anthony, C.D., and Hickerson, C.A.M., 2013. Assortative mating in a polymorphic salamander. Copeia 2013:676-683.

Anthony, C.D, Venesky, M.D., and Hickerson, C.A.M. 2008. Ecological separation in polymorphic terrestrial salamander. Journal of Animal Ecology 77:646-653.

Andersson, M. 1994. Sexual Selection. Princeton, New Jersey.

Atatalo, R.V., Lundberg, A., and Stahlbrandt, K. 1984. Female mate choice in the pied flycatcher *Ficedula hypoleuca.* Behavioral Ecology and Sociobiology 14:253-261.

Arak, A. 1988. Female mate selection in the natterjack toad: Active choice or passive attraction? Behavioral Ecology and Sociobiology 22:317–327.

Aspbury, A.S., and Basolo, A.L. 2002. Repeatable female preferences, mating order and mating success in the poeciliid fish, *Heterandria formosa*. Behavioral Ecology and Sociobiology 51:238–244.

Berglund, A., Bisazza, A., and Pilastro, A. 1996. Armaments and ornaments: An evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58:385-399.

Bradley, R.A., and Terry, M.E. 1952. Rank analysis on incomplete block designs 1: The method of paired comparisons. Biometrika 39:324-345.

Brown, J.L., Maan, M.E., Cummings, M.E., and Summers, K. 2010. Evidence for selection on coloration in a Panamanian poison frog: A coalescent-based approach. Journal of Biogeography. 37:891–901.

Boell, S., and Linsenmair, K.E. 1998. Size-dependent male reproductive success and size-assortative mating in midwife toad *Alytes obstetricans.* Amphibia-Reptilla 19:75-89.

Bunnell, P. 1973. Vocalizations in the territorial behavior of the frog *Dendrobates pumilio.* Copeia 1973:277-284.

Censky, E.J. 1997. Female mate choice in the non-territorial lizard *Ameiva plei* (Teiidae). Behavioral Ecology and Sociobiology 40:221-225.

Choudhury, S., and Black, J.M. 1993. Mate selection behavior and sampling strategies in geese. Animal Behaviour 46:747-757.

Christensen, R., and Kleindorfer, S. 2007. Assortative pairing and divergent evolution in Darwin's small tree finch, *Camarhynchus parvulus.* Journal of Ornithology 148:463-470.

Cooper, W.E., and Vitt, L.J. 1993. Female mate choice of large male broad-headed skinks. Animal Behaviour 45:683-693.

Conte, G.L., and Schluter, D. 2013. Experimental confirmation the body size determines mate preference via phenotype matching in a stickleback species. Evolution 67:1477-1484.

Crothers, L.R., Gering, E., and Cummings, M.E. 2011. Aposematic signal variation predicts male-male interactions in a polymorphic poison frog. Evolution 65:599-605.

Crothers L.R., and Cummings, M.E. 2013. Warning signal brightness variation: Sexual selection may work under the radar of natural selection in populations of a polytypic poison frog. The American Naturalist 181:E116-E124.

Crothers, L.R., and Cummings, M.E. 2015. A multifunctional warning signal behaves as an agonistic status signal in a poison frog. Behavioral Ecology 00:1-9.

Darst, C.R., Cummings, M.E., and Cannatella, D.C. 2006. A mechanism for diversity in warning signals: Conspicuousness versus toxicity in poison frogs. Proceedings of the National Academy of Sciences of the United States of America 103:5852–5857.

Dreher, C.E., and Pröhl, H. 2014. Multiple sexual signals: Calls over colors for mate attraction in an aposematic, color-diverse poison frog. Frontiers in Ecology and Evolution 2:22.

Donnelly, M.A. 1989. Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. Ecological Monographs 59:207-221.

Egger, B., Obermüller, B., Eigner, E., Sturmbauer, C., and Sefc, K.M. 2008. Assortative mating preferences between colour morphs of the endemic Lake Tanganyika cichlid genus *Tropheus*. Hydrobiologia 615:37-48.

Endler, J.A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41:315–352.

Fairbairn, D.J. 1988. Sexual selection for homogamy in the Gerridae: An extension of Ridley's comparative approach. Evolution 42:1212-1222.

Fawcett, T.W., and Johnstone, R.A. 2003. Mate choice in the face of costly competition. Behavioral Ecology 14:771-779.

Forester, D.C., and Czarnowsky, R. 1985. Sexual selection in the Spring Peeper, *Hyla crucifer* (Amphibia, Anura): Role of the advertisement call. Brill 92:112–128.

Gabor C. 1999. Association patterns of sailfin mollies (*Poecilia latipinna*): Alternative hypotheses. Behavioral Ecology and Sociobiology 46:333–340.

Guariguata, M.R., Chazdon, R.L., Denslow, J.S., Dupuy, J.M., and Anderson, L. 1997. Structure and floristics of secondary and old-growth forest stands in lowland Costa Rica. Plant Ecology 132:107–120.

Gould, J., and Gould, C. 1989. Sexual Selection. New York, N.Y.

Graham-Reynolds, R., and Fitzpatrick, B.M. 2007. Assortative mating in a poison-dart frog based on an ecologically important trait. Evolution 61:2253-2259.

Guevara-Fiore, P., Stapley, J., and Watt, P.J. 2010. Mating effort and female receptivity: How do male guppies decide when to invest in sex? Behavioral Ecology and Sociobiology 64:1665-1672.

Harari, A.R., Handler, A.M., and Landolt, P.J. 1999. Size-assortative mating, male mate choice and female choice in the curculinoid beetle *Diaprepes abbreviates.* Animal Behaviour 58:1191-1200.

Hegna, R.H., Saporito, R.A., Gerow, K.G., and Donnelly, M.A. 2011. Contrasting colors of an aposematic poison frog do not affect predation. Annales Zoologici Fennici 48:29–38.

Hill, G.E. 1990. Female house finches prefer colourful males: Sexual selection for a condition-dependent trait. Animal Behaviour 40:563-572.

Hill, G.E. 1991. Plumage coloration is a sexually selected indicator of male quality. Nature 350:337-339.

Hill, G.E., and Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. Proceeding of the Royal Society, London 258:47-52.

Hoefler, C.D. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidipus clarus*. Animal Behaviour 73:943-954.

Hoskin, C.J. 2004. Australian microhylid frogs (*Cophixalus* and *Austrochaperina)*: Phylogeny, taxonomy, calls, distribution and breeding biology. Australian Journal of Zoology 52:237-269.

Jawor, J.M., Linville, S.U., Beall, S.M., and Breitwish, R. 2003. Assortative mating by multiple ornaments in northern cardinals (*Cardinalis cardinalis).* Behavioral Ecology 14:515-520.

Johnson, J.B., and Basolo, A.L. 2003. Predator exposure alters female mate choice in the green swordtail. Behavioral Ecology 14:619-625.

Limerick, S. 1980. Courtship behavior and oviposition of the poison-arrow frog *Dendrobates pumilio.* Herpetologica 36:69-71.

Maan, M.E., and Cummings, M.E. 2008. Female preferences for aposematic signal components in a polymorphic poison frog. Evolution 62:2334-2345.

Maan, M.E., and Cummings, M.E. 2009. Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. Proceedings of the National Academy of Sciences, USA 106:19072-19077.

Meuche, I., Brusa, O., Linsenmair, K.E., Keller, A., and Pröhl, H. 2013. Only distance matters-non-choosy female in a poison frog population. Frontiers in Zoology 10:1-16.

Montgomerie, R. 2008. CLR, version 1.05. Queens's University, Kingston, Canada.

Noonan, K.C. 1983. Female mate choice in the cichlid fish, *Cichlasoma nigrofasciatum.* Animal Behavior 31:1005-1010.

Oneal, E., Connallon, T., and Knowles, L.L. 2007. Conflict between direct and indirect benefits of female choice in desert *Drosophila*. Biology Letters 3:29–32.

Pack, A.A., Herman, L.M., Spitz, S.S., Craig, A.S., Hakala, S., Keakos, M.H., Milette, E., Herman, E.Y.K., Carroll, E., Levitt, S., and Lowe, C. 2012. Size-assortative pairing and discrimination of potential mates by humpback whales in the Hawaiian breeding grounds. Animal Behaviour 84:983-993.

Paez, V., Bock, B., and Rand, A. 1993. Inhibition of evoked calling of *Dendrobates pumilio* due to acoustic interference from cicada calling. Biotropica 25:242–245.

Price, T. 1984. Sexual selection on body size, territory and plumage variables in a population of Darwin's Finches. Evolution 38:327–341.

Pröhl, H., and Hödl, W. 1999. Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio.* Behavioral Ecology and Sociobiology 6:215–220.

Pröhl, H. 2003. Variation in male calling behavior and relation to male mating success in the Strawberry Poison Frog (*Dendrobates pumilio*). Ethology 109:273-290.

Pröhl, H. 2005. Territorial behavior in dendrobatid frogs. Journal of Herpetology 39:354-365.

Rasband, W.S. 2014. ImageJ, U.S. National Institutes of Health, Bethesda, MD.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richards-Zawacki, C.L., Wang, I.J., and Summers, K. 2012. Mate choice and the genetic basis for colour variation in a polymorphic dart frog: Inferences from a wild pedigree. Molecular Ecology 21:3879–3892.

Ryan, M.J., Tuttle, M.D., and Rand, A. 1982. Bat predation and sexual advertisement in a Neotropical anuran. *The American Naturalist* 119:136–139.

Ryan, M.J., and Rand, A.S. 1990. The sensory bias of sexual selection for complex calls in the tungara frog. Evolution 44:305–314.

Savage, J.A. 2002. The amphibians and reptiles of Costa Rica: A herpetofauna between two continents, between two seas. Chicago, Illinois. pp 286-388.

Saporito, R.A., Zuercher, R., Roberts, M., Gerrow, K.G., and Donnelly, M.A. 2007a. Experimental evidence for aposematism in the poison frog *Oophaga pumilio*. Copeia 4:1006-1011.

Saporito, R.A., Donnelly, M.A., Norton, R.A., Garraffo, H.M., Spande, T.F., and Daly, J.W. 2007b. Oribatid mites as a major dietary source for alkaloids in poison frogs. Proceedings of the National Academy of Sciences 104:8885-8890.

Searcy, W.A. 1979. Female choice of mates: A general model for birds and its application to red winged Blackbirds (*Agelaius phoeniceus*). American Naturalist 114:77-100.

Siddiqi, A., Cronin, T.W., Loew, E.R., Vorobyev, M., and Summers, K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. Journal of Experimental Biology 207:2471-2485.

Staudt, K., Meneses-Ospina, S., Mebs, D., and Pröhl, H. 2010. Foraging behavior and territoriality of the strawberry poison frog (*Oophaga pumilio*) in dependence of the presence of ants. Amphibia Reptilia 31:217-227.

Stuart-Fox, D.M., Firth, D., Moussalli, A., and Whiting, M.J. 2006. Multiple signals in chameleon contests: Designing and analyzing animal contests as a tournament. Animal Behaviour 71:1263-1271.

Stynoski, J.L. 2009. Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog (*Oophaga pumilio*). Animal Behaviour 78:1351-1356.

Summers, K., Symula, R., Clough, M., and Cronin, T. 1999. Visual mate choice in poison frogs. Proceedings of the Royal Society of London, Biology 266:2141-2145.

Summers, K., Cronin, T.W., and Kennedy, T. 2003. Variation in spectral reflectance among populations of *Dendrobates pumilio*, the strawberry poison frogs with contrasting parental behavior. Journal of Heredity 88:35-53.

Thunken, T., Meuthen, D., Bakker, T., and Baldauf. S. 2012. A sex specific tradeoff between mating preference for genetic compatibility and body size in a cichlid fish with mutual mate choice. Proceeding of the Royal Society, Biology 279:2959-2964.

Traub, J. 2014. Quantification of color and pattern and a test of stabilizing selection in the strawberry poison frog *Oophaga pumilio* from the La Selva Biological Station in Costa Rica. Master's Thesis. John Carroll University.

Trivers, R.L. 1972. Parental investment and sexual selection. Chicago, Illinois. pp 137-179.

Turner, H., and Firth, D. 2012. Bradley-Terry Models in R: The BradleyTerry2 Package. Journal of Statistical Software 48:1-21.

Weatherhead, P.J. Prosser, M.R., Gibbs, H.L., and Brown, G.P. 2002. Male reproductive success and sexual selection in northern water snakes determined by microsatellite DNA analysis. Behavioral Ecology 13:808-815.

West-Eberhard, M. 1983. Sexual selection, social competition, and speciation. Quarterly Review of Biology 58:155-183.

Wilbur, H.M., Rubenstein, D.I., and Farichild, L. 1978. Sexual selection in toads: The roles of female choice and male body size. Evolution 32:264–270.

Wong, S., Parada, H., and Narins, P.M. 2009. Heterospecific Acoustic Interference: Effects on calling in *Oophaga pumilio*. Biotropica 41:74–80.

TABLE 1- All possible combinations of color, brightness, and pattern in a group of eight males used in the experimental study. Each male was used four times in eight different trials. Seven groups of males with these combinations were used for the experiment. Orange males were >0.47, red males were <0.42. Dull males had a total reflectance flux of <66 and bright males were >86. Pattern was estimated by an observer.

Male	Hue	Brightness	Pattern
1	Orange	Bright	Patterned
2	Orange	Bright	Not Patterned
3	Orange	Dull	Patterned
4	Orange	Dull	Not Patterned
5	Red	Bright	Patterned
6	Red	Bright	Not Patterned
7	Red	Dull	Patterned
8	Red	Dull	Not Patterned

TABLE 2- Descriptive statistics from 20 male frogs collected from the three sites to obtain "extreme" male cutoff points for each color metric.

			Range	
Location	**Color metric**	**Mean ± SD**	**Min**	**Max**
H	Hue	0.46 ± 0.01	0.36	0.61
	Brightness	68.04 ± 4.54	37.85	112.32
SP	Hue	0.48 ± 0.01	0.41	0.54
	Brightness	77.89 ± 3.94	42.24	112.54
STR	Hue	0.39 ± 0.01	0.28	0.46
	Brightness	82.35 ± 5.87	45.86	123.03

* H = Huertos; SP = Successional Plot; STR = Senderos Tres Rio 3950-4150.

TABLE 3- Results from the Bradley-Terry model for each analysis. Significant values are bolded.

Analysis type	Male trait	Estimate	Std.Err.	z-value	p
L1L2, R1R2 (time)*	Brightness	0.000	0.003	-0.070	0.945
	Hue	-0.269	1.218	-0.221	0.825
	Pattern	0.032	0.036	0.897	0.370
	SVL	-0.158	0.156	-1.014	0.311
	Mass	3.714	1.551	2.395	**0.017**
L1-L6, R1-R6 (time)*	Brightness	0.002	0.003	0.519	0.603
	Hue	-0.130	1.265	-0.103	0.918
	Pattern	0.017	0.037	0.458	0.647
	SVL	-0.259	0.162	-1.601	0.109
	Mass	4.484	1.611	2.784	**0.005**
Number of looks	Brightness	0.000	0.002	-0.262	0.793
	Hue	-0.008	1.060	-0.007	0.994
	Pattern	0.023	0.031	0.729	0.446
	SVL	-0.042	0.139	-0.299	0.765
	Mass	0.585	1.366	0.429	0.668
Intervals L1L2, R1R2*	Brightness	0.000	0.004	0.129	0.897
	Hue	-0.337	1.406	-0.240	0.810
	Pattern	0.009	0.041	0.211	0.833
	SVL	0.036	0.177	0.201	0.841
	Mass	1.396	1.771	0.788	0.431
Intervals all quads	Brightness	0.000	0.003	0.042	0.966
	Hue	-0.162	1.167	-0.139	0.889
	Pattern	0.003	0.034	0.937	0.349
	SVL	-0.288	0.149	-1.920	0.054
	Mass	3.749	1.485	2.524	**0.012**

* Refers to quadrants presented in FIG. 3.

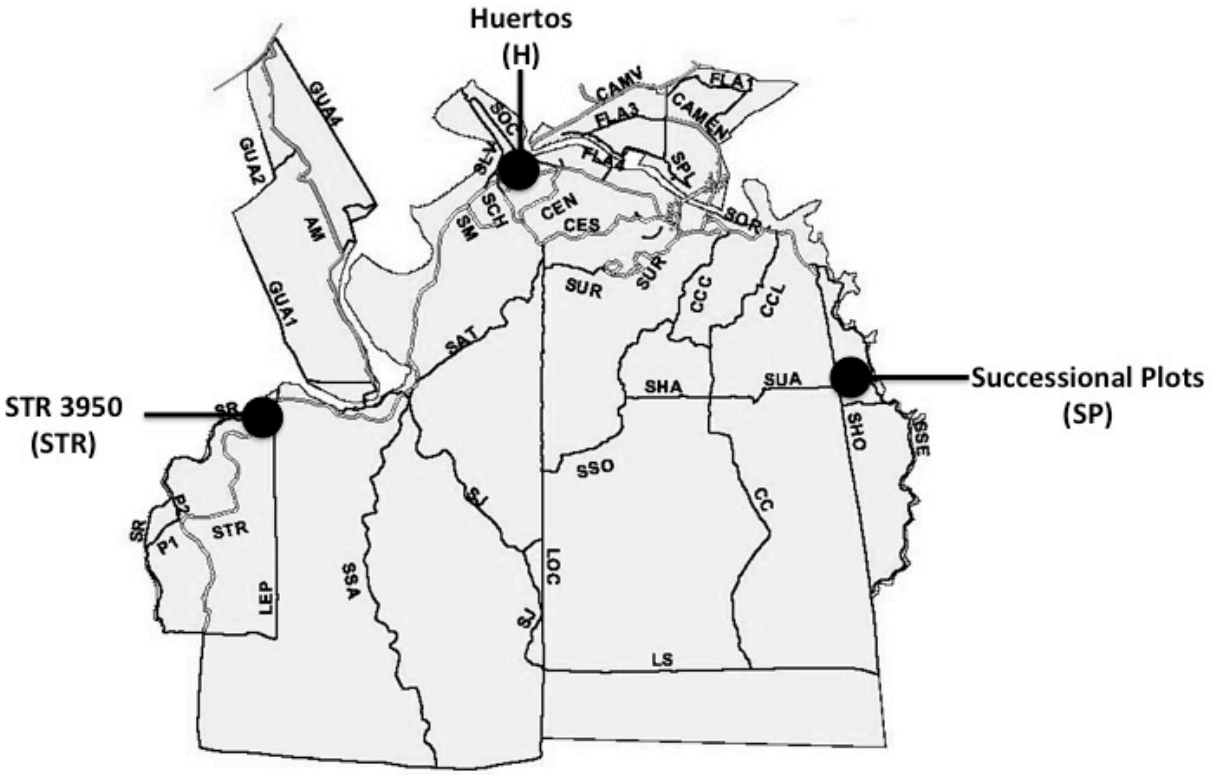

FIG. 1- La Selva Biological Research Station, showing the location of each field site used for frog collections. The major trails on the property are indicated by two/three letter abbreviations. For a key to the abbreviators see http://ots.ac.cr/.

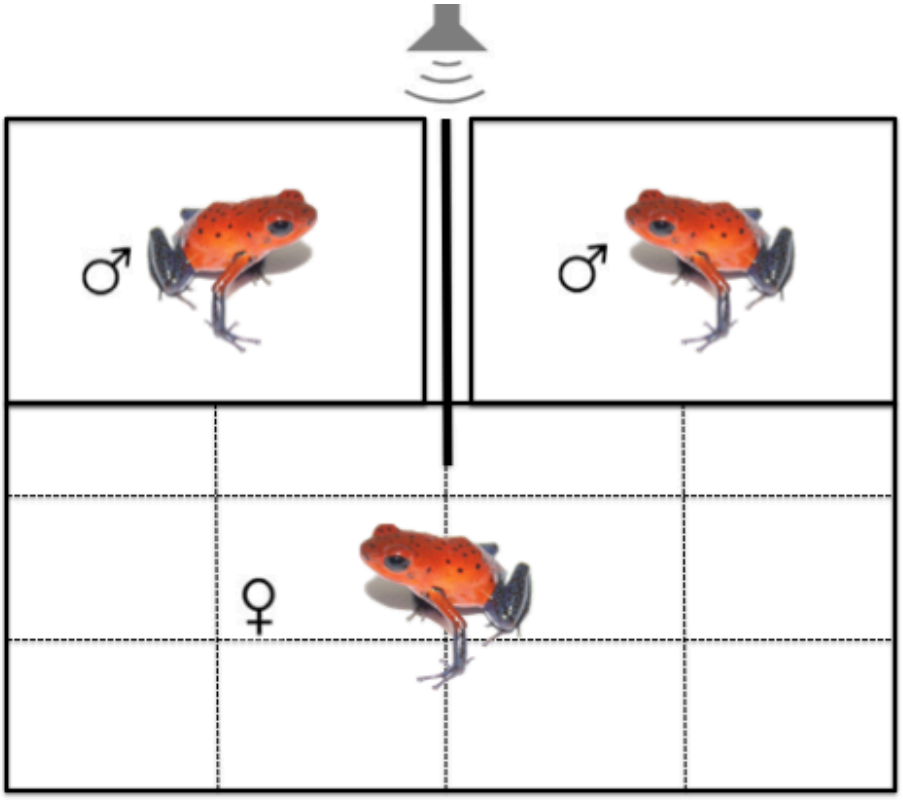

FIG. 2- Experimental arena used in mate choice trials. The speaker symbol behind the male chambers represents the placement of the speaker broadcasting cicada calls throughout each trial. The dark line between the male chambers represents the visual barrier between the males to prevent them from seeing each other. Lines on the floor of the female chamber represent the 12 quadrants used to track the location of the female. Refer to FIG. 3.

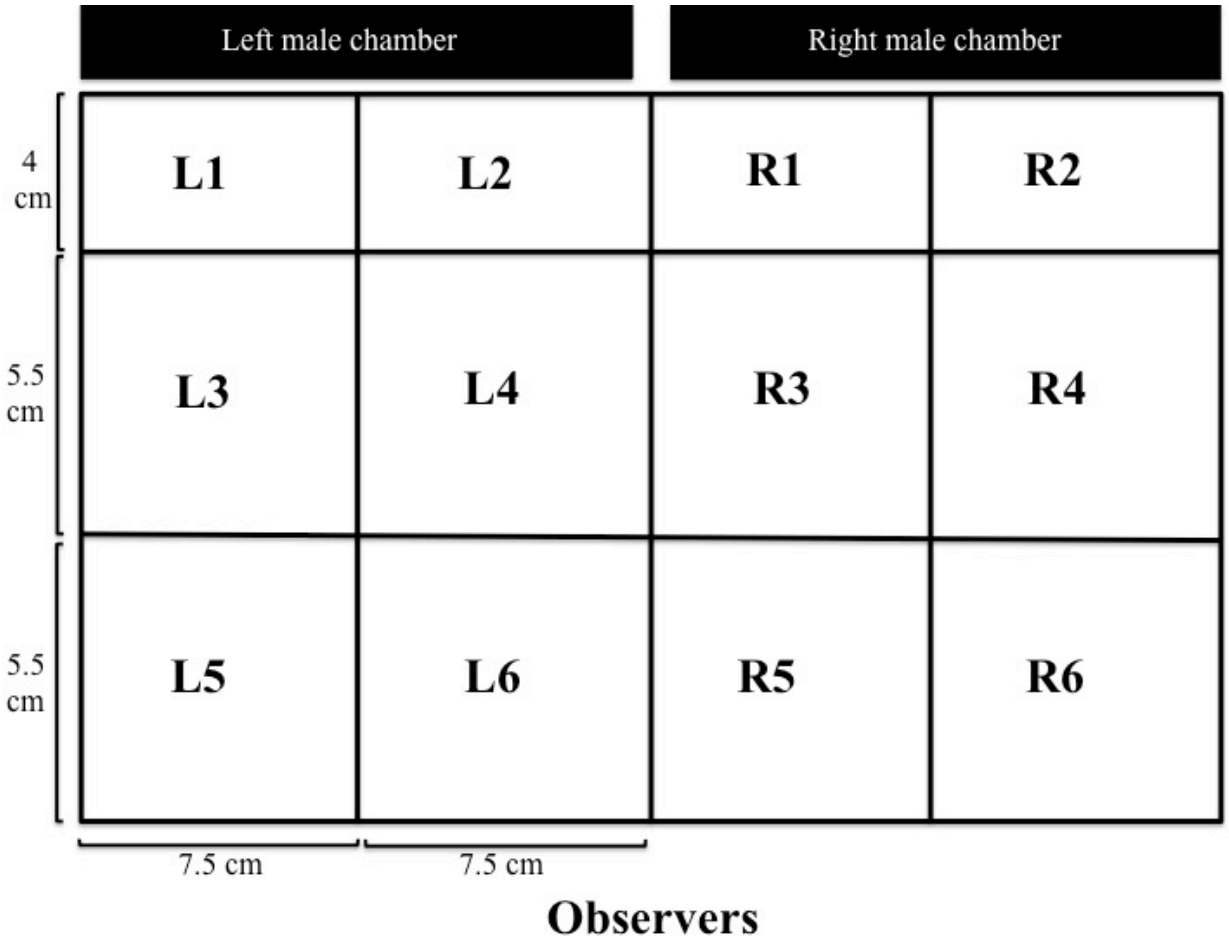

FIG. 3- Experimental arena floor gridding. “L” represents the left side, and “R” represents the right side. Females were allowed to move throughout L1-R6.

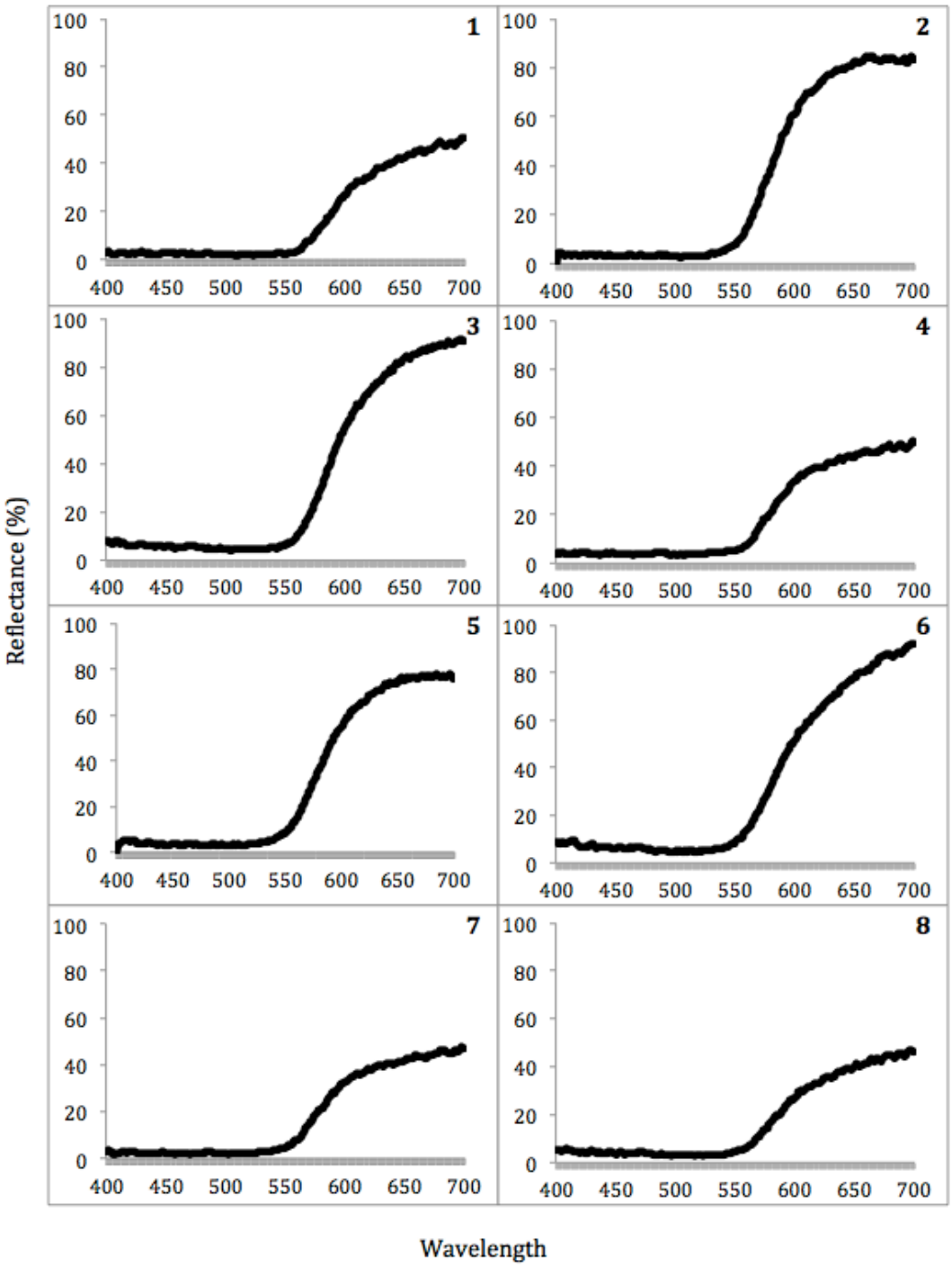

FIG. 4- Male reflectance spectra for each extreme male averaged for the 7 groups of 8 males. (1) **Male 1:** dull, red, patterned, (2) **Male 2:** bright, orange, patterned, (3) **Male 3:** bright, red, no pattern, (4) **Male 4:** dull, orange, no pattern, (5) **Male 5:** bright, orange, no pattern, (6) **Male 6:** bright, red, pattern, (7) **Male 7:** dull, orange, pattern, and (8) **Male 8:** dull, red, no pattern.

APPENDIX 1- Raw values of hue, brightness, and chroma for all males collected throughout the experiment. Bolded individuals are those that were used in the choice trials as an "extreme" male.

Location	ID Number	Hue	Brightness	Chroma
H	M-01	0.43	76.28	38.76
H	M-02	0.45	55.47	29.11
H	**M-03**	**0.48**	**57.14**	**30.31**
H	M-04	0.36	59.17	38.61
H	M-05	0.47	37.85	20.61
H	M-06	0.44	55.32	33.14
H	M-07	0.47	49.19	26.41
H	M-08	0.52	73.61	29.22
H	**M-09**	**0.41**	**51.32**	**29.88**
H	M-10	0.46	105.08	58.57
H	M-11	0.49	89.28	52.62
H	M-12	0.61	70.48	30.97
H	M-13	0.46	38.67	22.54
H	M-14	0.48	66.66	34.33
H	M-15	0.43	112.32	59.59
H	M-16	0.45	84.31	43.25
H	M-17	0.45	62.33	34.94
H	**M-18**	**0.40**	**92.28**	**53.51**
H	M-19	0.41	56.71	30.81
H	M-20	0.41	67.41	30.17
H	M-21	0.36	78.38	46.26
H	**M-22**	**0.40**	**56.58**	**37.06**
H	M-23	0.37	82.08	60.40
H	M-24	0.41	68.21	44.04
H	M-25	0.41	58.07	36.97
H	M-26	0.41	79.41	53.45
H	**M-27**	**0.37**	**92.57**	**55.47**
H	M-28	0.46	38.51	25.61
H	M-29	0.46	43.89	22.59
H	M-30	0.37	77.22	47.55
H	M-31	0.41	61.02	34.07
H	M-32	0.41	79.95	45.59
H	M-33	0.42	66.27	39.18
H	M-34	0.36	46.28	30.92
H	M-35	0.42	54.60	38.02

H	M-36	0.39	72.59	54.95
H	M-37	0.39	71.28	37.10
H	M-38	0.42	57.53	40.68
H	M-39	0.39	90.78	35.64
H	M-40	0.39	71.58	53.22
H	M-41	0.40	79.34	55.20
H	M-42	0.40	63.16	32.34
H	M-43	0.40	66.69	44.46
H	M-44	0.37	53.95	31.52
H	**M-45**	**0.36**	**104.79**	**75.73**
H	M-46	0.41	52.20	36.68
H	M-47	0.44	55.22	35.38
H	M-48	0.34	77.02	42.57
H	M-49	0.39	58.61	40.69
H	M-50	0.37	43.96	21.45
H	M-51	0.38	78.14	50.96
H	M-52	0.44	70.43	50.55
H	**M-53**	**0.41**	**114.21**	**80.73**
H	**M-54**	**0.39**	**55.72**	**30.36**
H	M-55	0.34	53.49	30.80
H	M-56	0.35	75.27	51.21
H	M-57	0.37	113.05	75.01
H	**M-58**	**0.30**	**44.48**	**27.78**
H	M-59	0.37	62.54	37.41
H	M-60	0.41	75.76	44.91
H	M-61	0.37	64.67	38.33
H	M-62	0.41	56.92	39.17
H	**M-63**	**0.37**	**52.67**	**32.73**
H	M-64	0.41	62.81	31.88
H	M-65	0.31	57.97	40.49
H	M-66	0.29	48.45	28.68
H	M-67	0.38	40.31	26.83
H	M-68	0.40	32.83	24.13
H	M-69	0.43	41.42	24.36
H	M-70	0.44	73.17	46.33
H	M-71	0.44	58.26	36.86
H	**M-72**	**0.47**	**65.16**	**42.24**
H	M-73	0.54	55.19	33.70
H	**M-74**	**0.34**	**42.86**	**31.73**
H	**M-75**	**0.39**	**46.34**	**28.32**
H	M-76	0.31	41.97	28.38
H	M-77	0.34	29.84	21.01

H	M-78	0.40	47.75	33.43
H	M-79	0.35	61.74	38.21
H	M-80	0.36	39.59	19.45
H	M-81	0.46	40.89	23.99
H	**M-82**	**0.43**	**66.56**	**44.70**
SP	M-01	0.45	88.41	49.07
SP	M-02	0.52	71.92	37.51
SP	M-03	0.53	59.99	34.21
SP	M-04	0.44	73.96	42.47
SP	M-05	0.49	42.24	23.18
SP	M-06	0.41	47.47	25.53
SP	**M-07**	**0.44**	**87.60**	**40.18**
SP	M-08	0.44	84.96	40.63
SP	M-09	0.48	87.78	43.14
SP	**M-10**	**0.49**	**56.03**	**35.75**
SP	**M-11**	**0.47**	**86.22**	**36.65**
SP	M-12	0.50	71.46	37.34
SP	M-13	0.49	95.26	66.15
SP	**M-14**	**0.49**	**77.39**	**49.83**
SP	M-15	0.53	79.35	46.18
SP	M-16	0.54	112.54	62.82
SP	**M-17**	**0.43**	**75.48**	**40.72**
SP	M-18	0.54	100.52	49.23
SP	M-19	0.52	92.77	47.03
SP	M-20	0.46	48.08	25.67
SP	**M-21**	**0.40**	**44.63**	**29.88**
SP	M-22	0.43	72.24	44.22
SP	M-23	0.40	46.79	29.37
SP	M-24	0.43	89.62	55.52
SP	M-25	0.44	63.16	44.75
SP	M-26	0.35	63.16	38.83
SP	M-27	0.41	75.45	49.57
SP	M-28	0.44	81.48	53.06
SP	M-29	0.34	92.27	57.57
SP	**M-30**	**0.42**	**65.72**	**48.59**
SP	M-31	0.42	101.79	74.38
SP	M-32	0.52	61.10	40.97
SP	**M-33**	**0.52**	**61.10**	**37.50**
SP	M-34	0.51	118.48	86.26
SP	**M-35**	**0.42**	**80.30**	**56.79**
SP	M-36	0.40	75.63	51.76
SP	M-37	0.42	73.61	54.51

SP	M-38	0.50	70.16	43.72
SP	M-39	0.32	100.26	58.13
SP	**M-40**	**0.43**	**75.73**	**43.36**
SP	M-41	0.38	78.89	56.58
SP	M-42	0.39	53.44	32.77
SP	M-43	0.43	116.13	71.82
SP	M-44	0.43	70.32	49.53
SP	M-45	0.37	54.98	31.61
SP	**M-46**	**0.45**	**63.84**	**35.73**
SP	M-47	0.47	52.03	30.02
SP	**M-48**	**0.44**	**144.98**	**91.93**
SP	M-49	0.39	68.46	38.82
SP	M-50	0.42	90.47	41.35
SP	M-51	0.41	71.58	49.70
SP	**M-52**	**0.47**	**73.64**	**44.44**
SP	M-53	0.40	56.36	38.74
SP	M-54	0.60	86.99	54.95
SP	**M-55**	**0.43**	**56.10**	**37.19**
SP	M-56	0.44	76.48	51.46
SP	M-57	0.48	80.30	52.48
SP	M-58	0.46	64.17	35.58
SP	**M-59**	**0.46**	**62.01**	**41.35**
SP	M-60	0.43	59.32	39.98
SP	M-61	0.42	65.40	46.90
SP	M-62	0.45	123.74	88.10
SP	**M-63**	**0.48**	**60.80**	**39.85**
SP	M-64	0.49	48.67	25.73
SP	**M-65**	**0.50**	**58.84**	**35.58**
SP	**M-66**	**0.49**	**62.56**	**42.68**
SP	M-67	0.46	103.32	73.36
SP	**M-68**	**0.35**	**44.74**	**29.48**
SP	M-69	0.38	98.44	57.47
SP	**M-70**	**0.42**	**51.65**	**34.13**
SP	M-71	0.44	68.36	43.22
SP	M-72	0.33	60.24	37.23
SP	M-73	0.47	122.86	68.14
SP	**M-74**	**0.37**	**43.58**	**24.45**
SP	M-75	0.48	56.57	37.38
SP	M-76	0.56	83.96	55.97
SP	M-77	0.70	63.19	32.10
SP	M-78	0.53	28.67	15.52
SP	M-79	0.52	98.51	66.49

SP	M-80	0.42	75.39	54.63
SP	M-81	0.41	40.40	25.79
SP	M-82	0.41	64.20	40.04
SP	M-83	0.39	66.22	42.91
SP	M-84	0.43	76.89	51.96
SP	M-85	0.52	46.89	29.30
SP	M-86	0.41	32.88	19.38
SP	M-87	0.40	79.09	58.35
SP	M-88	0.48	93.86	60.53
SP	**M-89**	**0.41**	**79.46**	**49.45**
SP	M-90	0.33	114.97	51.68
STR	**M-01**	**0.39**	**112.03**	**60.81**
STR	M-02	0.35	116.46	67.51
STR	**M-03**	**0.31**	**123.69**	**46.67**
STR	M-04	0.28	103.53	33.65
STR	M-05	0.43	71.46	43.01
STR	M-06	0.43	101.42	49.02
STR	M-07	0.42	64.27	31.46
STR	M-08	0.36	62.05	34.26
STR	M-09	0.45	62.47	39.05
STR	M-10	0.35	77.51	32.92
STR	M-11	0.39	52.24	22.70
STR	M-12	0.34	45.86	19.81
STR	M-13	0.40	56.41	26.18
STR	M-14	0.44	50.80	22.12
STR	M-15	0.40	54.53	25.02
STR	M-16	0.44	73.93	38.33
STR	M-17	0.46	104.66	65.37
STR	M-18	0.39	113.15	59.98
STR	M-19	0.45	85.54	49.34
STR	M-20	0.43	88.91	52.24
STR	**M-21**	**0.43**	**107.58**	**68.25**
STR	M-22	0.38	69.45	39.65
STR	M-23	0.48	78.11	35.74
STR	M-24	0.43	50.84	27.86
STR	M-25	0.46	57.82	42.48
STR	M-26	0.43	53.55	31.60
STR	M-27	0.42	68.15	44.13
STR	M-28	0.44	104.18	70.06
STR	M-29	0.47	54.31	24.18
STR	**M-30**	**0.45**	**69.16**	**40.08**
STR	M-31	0.45	96.89	48.73

STR	**M-32**	**0.38**	**78.89**	**58.29**
STR	M-33	0.39	49.16	29.13
STR	**M-34**	**0.34**	**57.62**	**34.70**
STR	**M-35**	**0.42**	**41.16**	**28.63**
STR	M-36	0.48	60.61	31.95
STR	**M-37**	**0.41**	**58.72**	**44.35**
STR	M-38	0.36	49.23	37.87
STR	M-39	0.34	65.95	46.46
STR	M-40	0.41	50.60	39.70
STR	M-41	0.45	83.28	54.64
STR	**M-42**	**0.38**	**76.95**	**58.08**
STR	M-43	0.43	47.15	33.16
STR	M-44	0.42	74.75	45.91
STR	M-45	0.34	61.41	33.56
STR	M-46	0.32	4.48	34.34
STR	M-47	0.40	71.40	42.02
STR	M-48	0.36	68.49	45.26
STR	M-49	0.34	41.14	20.01
STR	**M-50**	**0.34**	**73.31**	**48.61**
STR	M-51	0.48	85.77	58.16
STR	**M-52**	**0.44**	**96.96**	**69.71**
STR	M-53	0.37	71.68	40.01
STR	M-54	0.42	101.14	73.97
STR	**M-55**	**0.53**	**24.88**	**16.73**
STR	M-56	0.41	38.74	20.91
STR	M-57	0.41	43.12	28.17
STR	M-58	0.41	51.52	27.21
STR	M-59	0.47	33.97	25.53
STR	M-60	0.37	40.42	26.44
STR	M-61	0.38	42.26	22.57
STR	M-62	0.31	60.11	36.52
STR	M-63	0.44	69.72	46.35
STR	M-64	0.45	66.41	36.58
STR	M-65	0.34	42.13	26.75
STR	M-66	0.40	40.28	26.98
STR	M-67	0.34	58.43	38.56
STR	M-68	0.41	52.48	31.45
STR	M-69	0.37	27.21	20.23
STR	M-70	0.41	56.05	36.28
STR	M-71	0.38	79.03	47.29
STR	M-72	0.35	56.31	30.53
STR	M-73	0.37	81.92	43.16

STR	M-74	0.34	56.48	39.01
STR	M-75	0.38	51.68	37.57
STR	**M-76**	**0.42**	**88.28**	**55.32**
STR	**M-77**	**0.38**	**44.10**	**28.21**
STR	M-78	0.54	77.38	46.71
STR	**M-79**	**0.38**	**64.84**	**46.00**
STR	M-80	0.42	62.89	42.25
STR	M-81	0.43	35.51	24.41
STR	M-82	0.52	47.48	33.48
STR	**M-83**	**0.40**	**51.71**	**33.55**
STR	M-84	0.40	63.94	38.07
STR	M-85	0.40	59.13	41.18
STR	M-86	0.37	44.74	38.57
STR	M-87	0.46	66.87	46.23
STR	M-88	0.41	68.49	45.14
STR	M-89	0.41	54.49	39.56
STR	M-90	0.42	61.32	41.82
STR	M-91	0.40	60.53	41.01
STR	M-92	0.40	64.74	43.82
STR	M-93	0.44	58.26	39.56
STR	M-94	0.36	74.27	44.65
STR	M-95	0.44	45.95	30.17
STR	M-96	0.42	36.52	27.01
STR	M-97	0.41	60.87	45.06

* H = Huertos; SP = Successional plot; STR = Sendero Tres Rio 3150-4150.

COLOR ASSORATATIVE MATING IN A MAINLAND POPULATON OF THE STRAWBERRY POISON FROG, *OOPHAGA PUMILIO*

Abstract

Assortative mating, whereby individuals mate with those who share similar characteristics to themselves, is a common mating tactic of many different species. This mating strategy has the potential to impact the genotypes and phenotypes of populations and influence evolutionary outcomes. Assortative mating has been proposed as a mechanism in driving color polymorphism between polytypic populations of the strawberry poison frog, *Oophaga pumilio,* in Bocas del Toro, Panama. However, field studies investigating natural and realistic mating preferences within a population of *O. pumilio* are lacking. The present study observed natural mating pairs of *O. pumilio* at La Selva Biological Research Station in Costa Rica, and correlated each pairs color phenotype. Females were found to mate with males who had similar coloration to themselves, indicating color assortative mating is occurring within this population. Assortative mating may be beneficial for *O. pumilio* because it results in offspring that are most suited to their environment and likely leads to decreased predation pressure and increased mating success. Additionally, the color phenotype of these frogs is possibly under both sexual and natural selective pressures to optimize mating success and reduce predatory pressure. Further, this study investigated the relationships of male phenotypic components and courtship behaviors and found that brighter males with greater contrast and hue call from higher perches, possibly indicating a male's condition or level of territorial aggression. This study provides valuable insight into realistic mate selection tactics within a population of *O. pumilio,* and indicates that female mate choice is likely

multimodal in that it is dependent on multiple phenotypic and behavioral signals emitted by a male.

Introduction

Sexual selection driven by female mate choice is an important process in understanding the generation and maintenance of biological diversity (Andersson, 1994; Graham-Reynolds & Fitzpatrick, 2007). In female mate selection, females non-randomly choose a mate based on specific traits in an attempt to ultimately increase their offspring's fitness (Trivers, 1972). Females choosing specific mates can encourage reproductive isolation, and potentially lead to speciation (Crespi, 1989). Often, males exhibit conspicuous displays in an attempt to attract and ultimately mate with a female (Emlen & Oring, 1977; Ryan, Tuttle, & Rand, 1982). In numerous taxa, including lepidopterans (Nilsson & Forsman, 2003), birds (Hill, 1990; Jacobs et al., 2014; Zuk et al., 1990), fishes (Endler, 1980; Selz, 2014), and amphibians (Acord et al., 2013; Green, 1991; Summers et al., 1999), females tend to choose mates with specific, often conspicuous, traits that directly or indirectly indicate high quality and fitness.

Sexual selection is capable of operating alongside other selective forces, and can result in the evolution of certain mate attraction strategies. It may work in opposition to natural selection, such as when a trait makes an individual more susceptible to parasites or predators. There are also examples, however, when sexual selection can work in conjunction with natural selection, such as when a sexually selected trait allows for easier mate recognition, thus reducing the time individuals may be vulnerable to predators (Andersson, 1994; Mayr, 1972). For example, the sexual advertisement call of the tungara frog, *Physalaemus pustulosus*, should make males more obvious and vulnerable to predators; however, via natural and sexual selective pressures, a specific acoustic regime has evolved that attracts females while simultaneously reducing detection by

predatory bats (Rand & Ryan, 1981; Ryan et al., 1982). Additionally, crickets are known to adjust their mating strategies in response to the presence of parasitoid flies, which are able to detect male crickets by honing in on their mating calls (Cade, 1975). By adjusting the amount and timing of their calls, crickets are able to successfully attract mates as well as reduce their exposure to predation (Cade, 1975). In these examples, natural and sexual selective forces are both influencing the evolution of complex mechanisms used for attracting mates. Therefore, studying the interactions between selective forces and the specific characters involved in mating is necessary for a more complete understanding of the ecology and evolution of sexual selection.

The mating systems of many taxa are characterized by assortative mating, whereby individuals choose mates that share similar characteristics to themselves (Jiang et al., 2013). Assortment can occur based on age (Ferrer & Penteriani, 2003), behavior (Bollache et al., 2000; Weis et al., 2005), size (Christensen & Kleindorfer, 2007; Conte & Schluter, 2013; Harari et al., 1999), and coloration (Elmer et al., 2009; Houtman & Falls, 1994; Graham-Reynolds & Fitzpatrick, 2007). Color assortative mating is a common strategy in polymorphic populations whereby reproduction between specific phenotypes occurs more frequently than random mating (Karlin, 1978). Assortative mating can influence the genotype and phenotype of populations, maintain local adaptation in progeny, lead to greater variance in male fitness, and result in prezygotic isolation, which can even lead to speciation in some cases (Crespi, 1989).

Amphibians, especially those in the frog family Dendrobatidae, are an especially good system to investigate color assortative mating due to their salient coloration that may be used as a signal in choosing a mate. The conspicuous coloration of some

dendrobatid frogs has commonly been thought to evolve under the pressures of natural selection, as an aposematic trait to deter predators and warn them of the chemical defenses these frogs contain in their skin (Saporito et al., 2007). However, it appears that coloration simultaneously protects frogs from predation and functions as a signal to attract mates (Summers et al., 1999; Maan & Cummings, 2008).

There have been many studies on the role of coloration in driving sexual selection in the dendrobatid poison frog, *Oophaga pumilio*. In a small part of their geographic range in the Bocas del Toro archipelago of northwestern Panama, there are over 15 different polytypic populations that span the visual spectrum of color (Maan & Cummings, 2008; Summers et al., 1999). Experimental studies in which females were given a choice between males of the same population with similar coloration (native) or males from different populations and colors (naïve) have found that females generally associate with similarly colored native males (Graham-Reynolds and Fitzpatrick, 2007; Maan & Cummings, 2008; Summers et al., 1999). Collectively, these studies provide evidence that color-based assortative mating may have led to the diversity of coloration between different populations in this island system. However, there is no evidence that this type of assortment occurs in nature, as these different populations are isolated on islands separated by ocean (Dreher & Pröhl, 2014; Gerhardt, 1982). Therefore, field studies examining natural mating interactions within populations are needed to better understand specific reproductive strategies of this species (Richards-Zawacki et al., 2012).

While many of the studies on sexual selection in *O. pumilio* occur between the polytypic populations in Bocas del Toro, the majority of the geographic range of *O.*

pumilio lies on the mainland from southern Nicaragua along the Caribbean coast of Costa Rica and into northwestern Panama. Only a few studies have examined female mate choice both between and within mainland populations. These studies have found that females tend to choose males based largely on their call properties and proximity, which may be independent of their color phenotype (Dreher & Pröhl, 2014; Meuche et al., 2013). The activity, rate, and frequency of male advertisement calls are known to increase male mating success (Pröhl, 2003), and it seems likely that calling is an important signal in female mate choice (Meuche et al., 2013). However, Dreher and Pröhl (2014) suggest that females may primarily use advertisement calls in long-distance detection of males, whereas color may be used in close range assessment and final mate selection. The importance of color in female mate choice within populations of *O. pumilio* is not fully understood and requires further study.

The goal of the present study was to observe naturally occurring mating pairs of *O. pumilio* and quantify their hue, brightness, chroma, and pattern to determine if they are assortatively mating. It is hypothesized that females will assortatively mate, and choose males with similar coloration and patterning to themselves.

Methods

Study site- This field-based experiment was conducted at the Organization for Tropical Studies, La Selva Biological Research Station in northeastern Costa Rica (10°26' N, 83°59' W) from 26 June 2014 – 21 July 2014. Male and female pairs of *Oophaga pumilio* were observed and collected for color quantification from three locations: Huertos (H), Successional Plots (SP), and Sendero Tres Rios between trail markers 3950-4150 (STR).

These locations were chosen based on a previous study that found variation in hue, brightness, and pattern at each location (Traub, 2014).

Observations- Observations of naturally occurring mating pairs of *O. pumilio* were conducted daily from 0600-1200 and 1500-1700, when male calling and male/female courtship is most common (Gardner & Graves, 2005; Limerick, 1980; Willink et al., 2014). A total of 64 observations occurred among the three sites.

Males were identified by following the sound of their advertisement calls to their location. Once located, a 2m area surrounding the calling male was searched for the presence of a female. The observer stayed at least 1m away from the male while searching, to avoid interfering with natural behavior. If a female was present within 2m of the male, observations immediately began, and lasted for 15 minutes. The observer (Meaghan Gade or Michelle Hill) stood at least 2m from the pair to avoid disrupting natural interactions (Limerick, 1980; Willink et al., 2014). The total time a male spent calling, the total number of calling bouts (short periods of intense calling, followed by a short break), the direction a male was facing while calling (towards or away from the female), distance and direction males moved between call bouts, male perch type (substrate on which the male was calling from), male perch height, and the distance and direction a female moved were recorded for each trial.

Interactions between males and females were categorized into three types of events. An interaction was considered a "non-mating event" if a female moved farther than 2m away from a male during the 15-minute observation period, and did not return to within 2m of the male within 5 minutes. "Non-mating events" were not included in the analysis. An interaction was considered a "probable mating event" if a female stayed

within 1m of a calling male, and indicated interest by orienting her body towards the male for a majority of the observation period, or by moving in his direction following a series of male calls directly toward the female's position. When some combination of these behaviors were observed throughout the 15-minute observation period, both the male and female were captured, and taken back to the laboratory for measurements. Finally, an interaction was considered a "definite mating event" if a female stayed within 1m of a calling male and indicated interest by orienting her body towards him and moving in his direction. If, at any point, the male faced and moved away from the female, and she followed him for at least 3 seconds, it was considered a "definite mating event". Many times, a male would move away from the female and the female would not follow him, so he would return to her and continue to call. It was only considered a "definite mating event" when the female followed the male, because it was assumed that, at this point the female was following the male to an oviposition site (Limerick, 1980). This particular behavior was deemed the "follow behavior", and when it was observed, regardless of the time in the observation period, the male and female pair were captured and taken back to the lab for measurements.

The distinction between "probable" and "definite" mating events was made to allow for the inclusion of both a less rigorous measure of mating success, and a more definite measure of success. "Probable" mating events were justified by a number of reasons. First, during the course of this study, some interactions were observed for longer than 15 minutes (n = 7 pairs; observations = 16min to 66min). All of these interactions would have been considered "probable" mating events after the 15-minute threshold used in the present study. In all 7 interactions, however, the "follow" behavior was observed

after 15 minutes, and therefore, the interaction was categorized as a “definite mating pair”. Additionally, a previous study found that 85% of females who were in contact with one male on a given day ultimately chose that male as a mate (Meuche et al., 2013). Therefore, it was assumed that if a female stayed with a male for 15 minutes, she was likely to mate with him. Further, courtship in this species can last between 15 to 120 minutes (Savage, 2002), and the pairs in this study could have been found at any point during courtship. For these reasons, the behaviors used to categorize each “probable” mating event were considered an appropriate measure of mating success. The results from the “definite” mated pairs and the combined “definite + probable” mating events are discussed below.

Measurements- The hue, brightness, and chroma of all the males and females were measured using an Ocean Optics (Dunedin, FL) USB 4000 UV-VIS spectrophotometer, with a PX-2 pulsed xenon light source and a R400-7-SR reflectance probe with a 400μm core diameter. Color measurements were taken with the spectrophotometer held at a fixed angle of 90° and 6mm from the dorsum of the frogs. White standard measurements were taken between each individual frog using a Labsphere certified reflectance standard. Three random points along the dorsum of each frog were selected and three spectrophotometric readings were measured at each location. These nine measurements were averaged together to obtain an average dorsal coloration for each individual frog. Following the equations detailed by Endler (1990), hue, brightness, and chroma was calculated using the Java-based program CLR (version 1.05, Montgomerie, 2008).

Dorsal patterning was recorded for each male by photographing the dorsum of each individual using a Sony Cyber-shot point-and-shoot camera attached to a tripod, which was orientated 15cm above each frog. Each photo was analyzed using the computer program ImageJ (version 1.48, Rasband, 2014). The percent of the dorsum covered with pattern was calculated by dividing the sum of the area for all blotches (i.e., pattern) by the total dorsal area. Snout-to-vent length (SVL) was measured to the nearest 0.01mm using digital calipers and mass was measured using a Pesola PPS200 digital pocket scale to the nearest 0.01g.

Statistical Analysis- Canonical correlation analysis (CCA) was used to correlate hue, brightness, chroma, and pattern as a composite phenotype between males and females. Analyses were conducted on "definite" mated pairs and on "definite + probable" mated pairs. Additionally, using backwards selection multiple regression, color phenotypic metrics of male frogs were used to predict behavioral and environmental parameters involved in courtship, including perch height, the time of day male courtship and mating occurred, and total calling time within a 15 minute time period. Furthermore, linear regression was used to evaluate the relationship between perch height and total calling time of a male. Only males who were observed for the entire 15-minute trial were included in the total calling time analysis (n=28). All analyses were conducted in R (R Core Team, 2013). The alpha level for all statistical tests was 0.05.

Results

A total of 35 "definite" mating pairs (H = 13, SP = 12, STR = 10) and 29 "probable" mating pairs (H = 12, SP = 7, STR = 10) were observed and collected among the three

sites. Total reflectance curves for each mating pair (definite and probable) are in Appendix 1 and 2.

There was no significant canonical correlation between male and female "definite" or "definite + probable" mated pairs when hue, brightness, chroma, pattern, mass, and SVL were included in the model ("definite": Canonical correlation = 0.77, $F_{49,116.12} = 1.38$, $p = 0.08$; "definite + probable": Canonical correlation = 0.62, $F_{49,222.73} = 1.34$, $p = 0.08$). However, when mass and SVL were removed from the model, there was a strong, significant correlation in overall color and pattern phenotype between male and female "definite" mating pairs (Canonical correlation = 0.694, $F_{16,86.18} = 2.71$, $p = 0.001$; Figure 1), which is largely based on the contribution of chroma and brightness (Standardized canonical correlates: $chroma_{female} = 0.888$, $chroma_{male} = 0.626$; $brightness_{female} = 0.810$, $brightness_{male} = 0.428$). There was also a strong and significant correlation in overall phenotype between male and female "definite + probable" mating pairs (Canonical correlation = 0.563, $F_{16,150.34} = 2.49$, $p = 0.002$), which is also largely based on the contribution of chroma and brightness (Standardized canonical correlates: $chroma_{female} = 0.788$, $chroma_{male} = 0.815$; $brightness_{female} = 0.810$, $brightness_{male} = 0.710$).

On the basis of the multiple regression model, hue, brightness, and chroma were significant positive predictors of the perch height of a male, with chroma having the largest impact on this relationship (Table 1). Backwards elimination analysis found that only mass was a significant predictor of the time of day a male courted and mated with a female, with larger males courting later in the day (after 1500 h; $t_{64,1} = 11.29$, $R^2 = 0.15$, $p = 0.001$). There were no significant phenotypic predictors for the total calling time of a

male during courtship (Table 2). Further, there was no significant relationship between male perch height and total calling time ($t_{26,1} = 1.31$, $R^2 = 0.05$, $p = 0.26$).

Discussion

In natural mating interactions within a mainland population of *O. pumilio,* females were found to pair with males that look most similar to themselves with respect to color. These findings provide the first evidence of assortative mating within a population of *O. pumilio* based on dorsal coloration, suggesting that mating in this species is not random, but instead involves color-based selection by females. Assortative mating has been previously reported between different populations of *O. pumilio* in the Bocas del Toro archipelago, but this is one of the first studies to find that assortative mating is likely occurring within a single population. Moreover, this study suggests that multiple selective forces may drive coloration in this frog, and that female mate choices are likely influenced by a variety of phenotypic and behavioral signals exhibited by males.

Assortative mating is a common mating strategy that has the potential to increase offspring fitness by increasing the relatedness among kin and perpetuate altruistic traits (Graham-Reynolds and Fitzpatrick, 2007; Jiang et al. 2013; Thiessen & Gregg, 1980). This mating strategy can preserve well-adapted traits, and offer stability in a constant environment (Wolf & Figueredo, 2011). The coloration of *O. pumilio* is one the most salient and obvious phenotypic features of these frogs, and seems to function in sexual selection. When female *O. pumilio* choose a male with a similar phenotype, the resulting offspring is likely one that is most suited to its environment and has less predatory pressures because the offspring's phenotype has undergone purifying selection to

represent the most common and advantageous color morph in a population (Graham-Reynolds & Fitzpatrick, 2007). Experimental evidence of stabilizing selection by way of predation has been shown to occur in the same population examined in the present study, whereby model replicas of *O. pumilio* with less common color morphs were selected out of the population (Paluh et al., 2014; Stuart et al., 2012; Traub, 2014). Therefore, if an intermediately colored (and therefore less common) offspring is produced as a result of a random, non-assortative mating event, it might be expected to experience higher predatory pressures. It may therefore be most beneficial to mate with an individual of similar phenotype, in order to decrease hybrid offspring potential and ultimately increase offspring survivorship. Furthermore, assortative mating may increase the mating success of progeny, because as individuals mate assortatively, the proportion of that "purified" phenotype increases, leading to more opportunities for future generations to successfully mate in an assortative manner (Jiang et al., 2013). By mating assortatively, females produce offspring that are most likely to survive by exhibiting traits that have previously been beneficial to survival and mating success in their local environment.

The mating preferences of *O. pumilio* have been well studied in the Bocas del Toro archipelago in Panama by examining mate choices between different polytypic populations. Summers et al. (1999) compared males from the orange, non-spotted Cayo Nancy population to individuals from the green, non-spotted Isla Popa population under various lighting conditions, and found evidence of assortative mating based on color alone. Graham-Reynolds and Fitzpatrick (2007) built upon Summers et al. (1999) by studying different populations that varied in color and pattern, and found that *O. pumilio* assortatively mate based on both of these phenotypic attributes. Further, Maan and

Cummings (2008) used multiple populations from Bocas del Toro and also found that females tend to choose mates similar in color to them when given the option between a similar colored male from the same population and a dissimilar colored male from a different population. Although these experiments do not represent pairings that would naturally occur in nature, these previous studies collectively illustrate that females tend to prefer to pair with their own color morph when given dissimilar options. However, one of the choice experiments in the Maan and Cummings (2008) laboratory study tested female choice between males and females of similar phenotypes, but from different locations. This is the only other study that has attempted to investigate female mate choices between males with limited phenotypic variation, to represent what would likely be found within a population. Maan and Cummings (2008) did not detect assortative mating between similarly colored males, which is in contrast to the results of the present study. It is possible that in the Maan and Cummings (2008) experiment, while the coloration of the two males were relatively similar, the size or behavioral differences between the males from different locations may have been enough for the females to detect, and therefore not mate assortatively. The present study supports and complements the more broad findings from Bocas del Toro, and provides evidence that females prefer a mate that is similarly colored when choosing between mates that are biologically available within their own population. Color assortative mating appears to be a common mechanism by which female's appear to choose mates in this species.

Mated pairs of males and females were most similar to each other in phenotype, and in particular, brightness and chroma. It has been found that brightness and chroma are tightly linked color components (Endler, 1990; LeBas & Marshall, 2000), and the

results from the present study support the idea that these traits are generally coupled. However, patterning was the least important phenotypic component with respect to female mate choice. Other studies have shown that dorsal patterning is not important in sexual selection (Maan & Cummings, 2008). Instead, patterning may be involved in predator deterrence and the aposematic function of color; however, there is little support for this hypothesis (Aronsson & Gamberale-Stille, 2008; Hegna et al., 2011). Therefore, further studies are needed to understand the primary function of dorsal patterning.

It is possible that multiple selective pressures may influence the phenotype of *O. pumilio.* In addition to serving a role in female mate selection, the conspicuous coloration of these frogs has been shown to act as an aposematic signal, driven by natural selection by way of predation pressure (Ruxton et al., 2005; Saporito et al., 2007). A synergistic effect between sexual and natural selection has been observed in other taxa such as the guppy *Poecilia reticulata*, whereby color patterning and the degree of iridescence is influenced by both mate attraction and predator avoidance (Endler, 1980). Additionally, the tungara frog, *Physalaemus pustulosus,* has evolved a complex advertisement call that straddles both attracting females and avoiding predation by bats that use frog calls to locate males (Rand & Ryan, 1981). The coloration of *O. pumilio* may be shaped by both natural and sexual selection to optimize the frog's survival and mating success.

While different selective pressures may act on this frog's phenotype as a whole, it is also possible that separate components of color are under different selective forces. The results from the current study suggest that brightness and chroma are the most important components driving mating assortment. Additionally, considering that birds, which are presumed to be one of the major predators of *O. pumilio* (Saporito et al., 2007; Paluh et

al., 2014), seem incapable of discriminating differences in the brightness among these frogs (Cummings & Crothers, 2013), it is likely that these two particular color components (i.e., brightness & chroma) are driven mainly by sexual selection. Hue may more strongly be influenced by natural selection because the current study found that hue had the least importance with respect to assortment. Additionally, clay model studies aimed at assessing predation pressure upon *O. pumilio* at La Selva have provided evidence for stabilizing selection by bird predators on frogs with variable hues (commonly referred to as color; Stuart et al., 2012; Traub, 2014). Studies on other dendrobatids have found novel color morphs to be selected by predators more often than local morphs, further suggesting that natural and stabilizing selection are acting on the color (hue) of these frogs (Amezquita et al., 2013; Chouteau & Angers, 2011; Noonan & Comeault, 2009). The findings of the present study, coupled with those of previous studies, suggest that brightness and chroma are driven mainly by sexual selection and hue is more strongly driven by natural selection as an aposematic trait.

While our results suggest the importance of color phenotype on female mate choice, it is likely there is a suite of traits and behaviors females use to assess a mate. Previous studies have indicated the importance of calling to female mate choice (Meuche et al., 2013; Pröhl, 2003). Calling may indicate greater fitness due to the energy investment involved in calling (Grafe, 1996; Taigen & Wells, 1985), or also may act as a way for females to detect a male from a distance in the dense forests these frogs inhabit (Meuche et al., 2013; Pröhl, 2003). Once a male is detected and approached, females may then use coloration cues for closer evaluation of males (Meuche et al. 2013). Additionally, the present study found that females seem to be evaluating all parts of a

male's phenotype (hue, brightness, chroma, & pattern), and are therefore using multiple cues to assess mates. Finally, courtship in this species is a long and complex event involving many behaviors and intricate interactions between males and females (Limerick, 1980; Savage, 2002). This courtship event as a whole may play a large role in female mate choice, in addition to her assessment of male phenotype. Female mate choice seems to be multimodal and based on a series of different variables exhibited by the males.

Male phenotype and courtship behaviors- In the present study, brighter males with greater contrast (chroma) and hue tended to call from higher perches. Crothers et al. (2011) suggested that males exhibiting a bright contrasting phenotype might be capable of sitting on higher perches because they are potentially sending stronger aposematic signals and are therefore less vulnerable to predators. Additionally, brighter males may sit on higher perches as a show of territorial aggression and greater calling ability (Meuche et al., 2013). Brighter males tend to be more aggressive and better defenders of their territory, which contain food and reproductive resources that are important to a female (Crothers et al., 2011; Crothers & Cummings, 2015; Donnelly, 1989). Additional research is needed to test these hypotheses, and determine whether or not brighter males and higher perches are more likely to mate with females. Previous studies have found that the amount of calling activity, which is the most important acoustic component in male mating success, is correlated with the average perch height in this species (Pröhl & Hödl, 1999; Pröhl, 2003). In the present study, however, there was no correlation between perch height and total calling time. Finally, the present study found that larger males court females later in the day. Larger males may be in better condition than smaller

males, because they are more adept at foraging and obtaining more energy, and therefore capable of courting throughout the day.

Conclusions- This study evaluated biologically relevant mate choices within a population of *O. pumilio* in a natural setting, and found that color phenotype-based assortative mating is occurring within this mainland population. A complex interaction between natural and sexual selection may be occurring whereby sexual selection is acting more heavily on brightness and chroma, while natural selection via predation is driving the hue of males. This study, coupled with others, indicates that a large suite of male characteristics and behaviors are responsible for female mating choices. This study provides valuable insight into realistic female mating decisions.

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Literature Cited

Acord, M.A., Anthony, C.D., & Hickerson, C.A.M. (2013). Assortative mating in a polymorphic salamander. *Copeia, 2013*, 676-683.

Amezquita, A., Castro, L., Arias, M., Gonzalez, M., & Esquivel, C. (2013). Field but not lab paradigms support generalization by predators of aposematic polymorphic prey: The *Oophaga histrionica* complex. *Evolutionary Ecology, 27*, 769-782.

Andersson, M. (1994). *Sexual Selection.* Princeton, New Jersey: Princeton University Press.

Aronsson M., & Gamberale-Stille, G. (2008). Domestic chicks primarily attend to colour, not pattern, when learning an aposematic coloration. *Animal Behaviour, 75*, 417–423.

Bollache, L., Gambade, G., & Cezilly, F. (2000). The influence of micro-habitat segregation on size assortative pairing in *Gammarus pulex. Archives fur Hydrobiologie, 147*, 547-558.

Cade, A. (1975). Acoustically orienting parasitoids: Fly phonotaxis to cricket song. *Science, 190,* 1312–1313.

Christensen, R., & Kleindorfer, S. (2007). Assortative pairing and divergent evolution in Darwin's small tree finch, *Camarhynchus parvulus. Journal of Ornithology, 148*, 463- 470.

Chouteau, M., & Angers, B. (2011). The role of predators in maintaining the geographic organization of aposematic signals. *The American Naturalist, 178*, 810-817.

Conte, G.L., & Schluter, D. (2013). Experimental confirmation the body size determines mate preference via phenotype matching in a stickleback species. *Evolution, 67*, 1477-1484.

Crespi, B.J. (1989). Causes of assortative mating in arthropods. *Animal Behaviour, 1*, 980–1000.

Crothers, L.R., Gering, E., & Cummings, M.E. (2011). Aposematic signal variation predicts male-male interactions in a polymorphic poison frog. *Evolution, 65*, 599-605.

Crothers, L.R., & Cummings, M.E. (2015). A multifunctional warning signal behaves as an agonistic status signal in a poison frog. *Behavioral Ecology, 00,* 1-9.

Cummings, M.E., & Crothers, L.R. (2013). Interacting selection diversifies warning signals in a polytypic frog: An examination with the strawberry poison frog. *Evolutionary Ecology, 27,* 693–710.

Donnelly, M.A. (1989). Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. *Ecological Monographs 59*, 207-221.

Dreher, C.E, & Pröhl, H. (2014). Multiple sexual signals: Calls over colors for mate attraction in an aposematic, color-diverse poison frog. *Frontiers in Ecology and Evolution, 2,* 22.

Elmer, K.R., Lehtonen, T.K., & Meyer, A. (2009). Color assortative mating contributes to sympatric divergence of a neotropical cichlid fish. *Evolution, 63*, 2750-2757.

Emlen, S.T., & Oring, L.W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*, 215-223.

Endler, J.A. (1980). Natural selection on color patterns in *Poecilia reticulata. Evolution, 34*, 76-91.

Endler, J.A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society*, *41,* 315–352.

Ferrer, M., & Penteriani, V. (2003). A process of pair formation leading to assortative mating: Passive age assortative mating by habitat heterogeneity. *Animal Behaviour, 66*, 137-143.

Gardner, E.A., & Graves, B.M. (2005). Responses of resident male *Dendrobates pumilio* to territory intruders. *Journal of Herpetology, 39*, 248–253.

Gerhardt, H.C. (1982). Sound pattern recognition in some North American treefrogs (Anura: Hylidae): Implications for mate choice. *American Zoologist, 22*, 581-595.

Graham-Reynolds, R., & Fitzpatrick, B.M. (2007). Assortative mating in a poison-dart frog based on an ecologically important trait. *Evolution,* 61, 2253-2259.

Grafe, T.U. (1996). Energetics of vocalization in the African reed frog (*Hyperolius marmoratus*). *Comparative Biochemistry and Physiology, 114*, 235-243.

Green, A.J. (1991) Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. *Animal Behaviour, 41,* 367-369.

Harari, A.R., Handler, A.M., & Landolt, P.J. (1999). Size assortative mating, male choice and female choice in the curculionid beetle *Diaprepes abbreviates. Animal Behaviour, 58*, 1191-1200.

Hegna, R.H., Saporito, R.A., Gerow, K.G., & Donnelly, M.A. (2011). Contrasting colors of an aposematic poison frog do not affect predation. *Annales Zoologici Fennici, 48*, 29–38.

Hill, G.E. (1990). Female house finches prefer colourful males: Sexual selection for a condition-dependent trait. *Animal Behaviour, 40*, 563-572.

Houtman, A.M., & Falls, J.B. (1994). Negative assortative mating in the white-throated sparrow, *Zonotrichia albicollis*: The role of mate choice and intra-sexual competition. *Animal Behaviour, 48*, 377-383.

Jacobs, A.C., Fair, J.M., & Zuk, M. (2014). Coloration, paternity, and assortative mating in Western Bluebirds. *Ethology*, *120*, 1-11.

Jiang, Y., Bolnick, D.I., & Kirkpatrick, M. (2013). Assortative mating in animals. *The American Naturalist, 181*, 125–38.

Karlin, S. (1978). Comparisons of positive assortative mating and sexual selection models. *Theoretical Population Biology, 14*, 281-312.

Lebas, N.R., & Marshall, N.J. (2000). The role of colour in signaling and male choice in the agamid lizard *Ctenophorus ornatus*. *Proceedings of the Royal Society: Biological Sciences*, *267*, 445–452.

Limerick, S. (1980). Courtship behavior and oviposition of the poison-arrow frog *Dendrobates pumilio. Herpetologica, 36*, 69-71.

Maan, M.E., & Cummings, M.E. (2008). Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution, 62*, 2334-2345.

Mayr, E. 1972. Sexual selection and natural selection. IN: *Sexual Selection and the Decent of Man*. Aldine: Chicago, IL, USA.

Meuche, I., Brusa, O., Linsenmair, K.E., Keller, A., & Pröhl, H. (2013). Only distance matters- non-choosy female in a poison frog population. *Frontiers in Zoology, 10*, 29.

Montgomerie, R. (2008). CLR, version 1.05. Queens's University, Kingston, Canada.

Noonan, B.P., & Comeault, A.A. (2009). The role of predator selection on polymorphic aposematic poison frogs. *Biology Letters, 5*, 51-54.

Nilsson, M., & Forsman, A. (2003). Evolution of conspicuous colouration, body size and gregariousness: A comparative analysis of lepidopteran larvae. *Evolutionary Ecology, 17*, 51–66.

Paluh, D.J., Hantak, M.M., & Saporito, R.A. (2014). A test of aposematism in the dendrobatid poison frog *Oophaga pumilio:* The importance of movement in clay model experiments. *Journal of Herpetology, 48,* 249-254.

Pröhl, H., & Hödl, W. (1999). Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behavioral Ecology and Sociobiology, 46*, 215–220.

Pröhl, H. (2003). Variation in male calling behavior and relation to male mating success in the Strawberry Poison Frog (*Dendrobates pumilio*). *Ethology, 109*, 273-290.

Rand, A.S., & Ryan, M.J. (1981). The adaptive significance of a complex vocal repertoire in a neotropical frog. *Zoology Tierpsychology, 57*, 209–214.

Rasband, W.S. (2014). ImageJ, U.S. National Institutes of Health, Bethesda, MD.

R Core Team. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richards-Zawacki, C.L., Wang, I.J., & Summers, K. (2012). Mate choice and the genetic basis for colour variation in a polymorphic dart frog: Inferences from a wild pedigree. *Molecular Ecology*, *21*, 3879–3892.

Ryan, M.J., Tuttle, M.D., & Rand, A. (1982). Bat predation and sexual advertisement in a neotropical anuran. *The American Naturalist, 119*, 136–139.

Ruxton, G.D., Sherratt, T.N., & Speed, M.P. (2005). *Avoiding attack: evolutionary ecology of crypsis, warning signals and mimicry*. Oxford, UK: Oxford University Press.

Saporito, R.A., Zuercher, R., Roberts, M., Gerow, K.G., & Donnelly, M.A. (2007). Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia*, *2007*, 1006–1011.

Savage, J.M. (2002). *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between two continents, between two seas*. Chicago, Il: University of Chicago.

Selz, O.M., Pierotti, M.E.R., Maan, M.E., Schmid, C., & Seehausen, O. (2014). Female preference for male color is necessary and sufficient for assortative mating in two cichlid sister species. *Behavioral Ecology, 25*, 612–626.

Stuart, Y.E., Dappen, N., & Losin, N. (2012) Inferring predator behavior from attack rates on prey-replicas that differ in conspicuousness. *PLOS ONE, 7,* 1-7.

Summers, K., Symula, R., Clough, M., & Cronin, T. (1999). Visual mate choice in poison frogs. *Proceedings of the Royal Society of London, Biology, 266*, 2141-2145.

Taigen, T.L., & Wells, K.D. (1985). Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *Journal of Comparative Physiology, 155*, 163-170.

Thiessen, D., & Gregg, B. (1980). Human assortative mating and genetic equilibrium: An evolutionary perspective. *Ethology and Sociobiology, 1*, 111-140.

Traub, J.T. (2014). *Quantification of color and pattern and a test of stabilizing selection in the strawberry poison frog Oophaga pumilio from the La Selva Biological Research Station in Costa Rica* (Master's Thesis). University Heights, OH: John Carroll University.

Trivers, R.L. (1972). *Parental investment and sexual selection.* Chicago, Illinois. pp 137-179.

Weis, A.E., Winterer, J., Vacher, C., Kossler, T.M., Young, C.A., & LeBuhn, G.L. (2005). Phenological assortative mating in flowering plants: the nature and consequences of its frequency dependence. *Evolutionary Ecology Research, 7*, 161-181.

Willink, B., Bolaños, F., & Pröhl, H. (2014). Conspicuous displays in cryptic males of a polytypic poison-dart frog. *Behavioral Ecology and Sociobiology, 68,* 249-261.

Wolf, P.S.A., & Figueredo, A.J. (2011). Fecundity, offspring longevity, and assortative mating: Parametric tradeoffs in sexual and life history strategy. *Biodemography and Social Biology, 57*, 171–183.

Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., & Costin, C. (1990). The role of male ornaments and courtship behavior in female mate choice of red jungle fowl. *The American Naturalist, 4*, 459–473.

Table 1. Multiple regression analysis using backwards elimination of male perch height and phenotypic components: (a) overall model, (b) each predictor variable.

(a)	R^2	t	df_d	df_n	P
	0.19	3.64	61	4	0.01
(b)	**β**	**β standard**	**t**	**df**	**P**
Hue	106.72	0.24	2.1	61	0.04
Brightness	-1.36	-0.99	-2.43	61	0.01
Chroma	2.15	1.01	2.67	61	0.01
SVL	-8.62	-0.49	-2.14	61	0.04

Table 2. Multiple regression analysis using backwards elimination of the total calling time in 15 minutes and phenotypic components of males: (a) overall model, (b) each predictor variable.

(a)	**R^2**	**t**	**df_d**	**df_n**	**p**
	0.11	0.45	21.00	6.00	0.84
(b)	**β**	**β standard**	**t**	**df**	**p**
Hue	3.95	0.22	0.32	21.00	0.75
Brightness	0.11	1.94	0.79	21.00	0.44
Chroma	-0.21	-2.74	-1.11	21.00	0.28
Pattern	0.07	0.19	0.28	21.00	0.78
Mass	-1.09	-0.06	-0.07	21.00	0.95
SVL	0.89	0.57	0.63	21.00	0.53

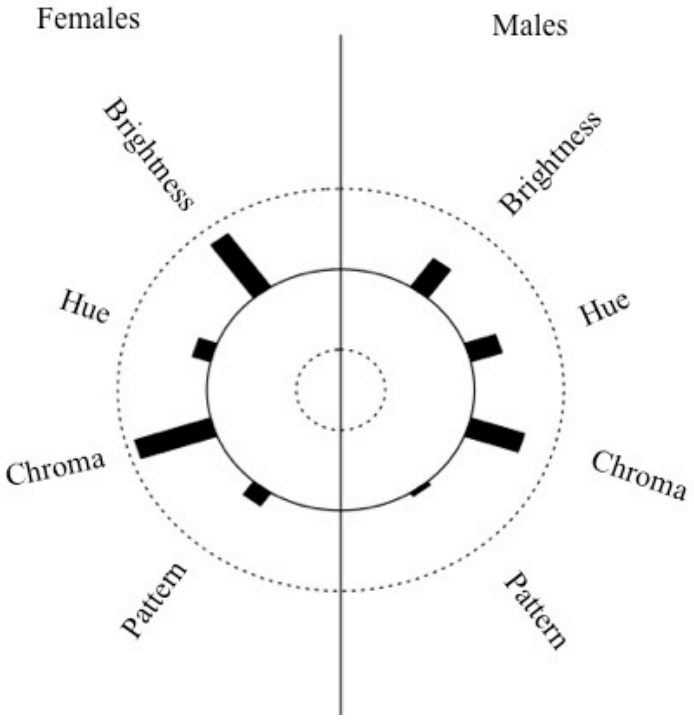
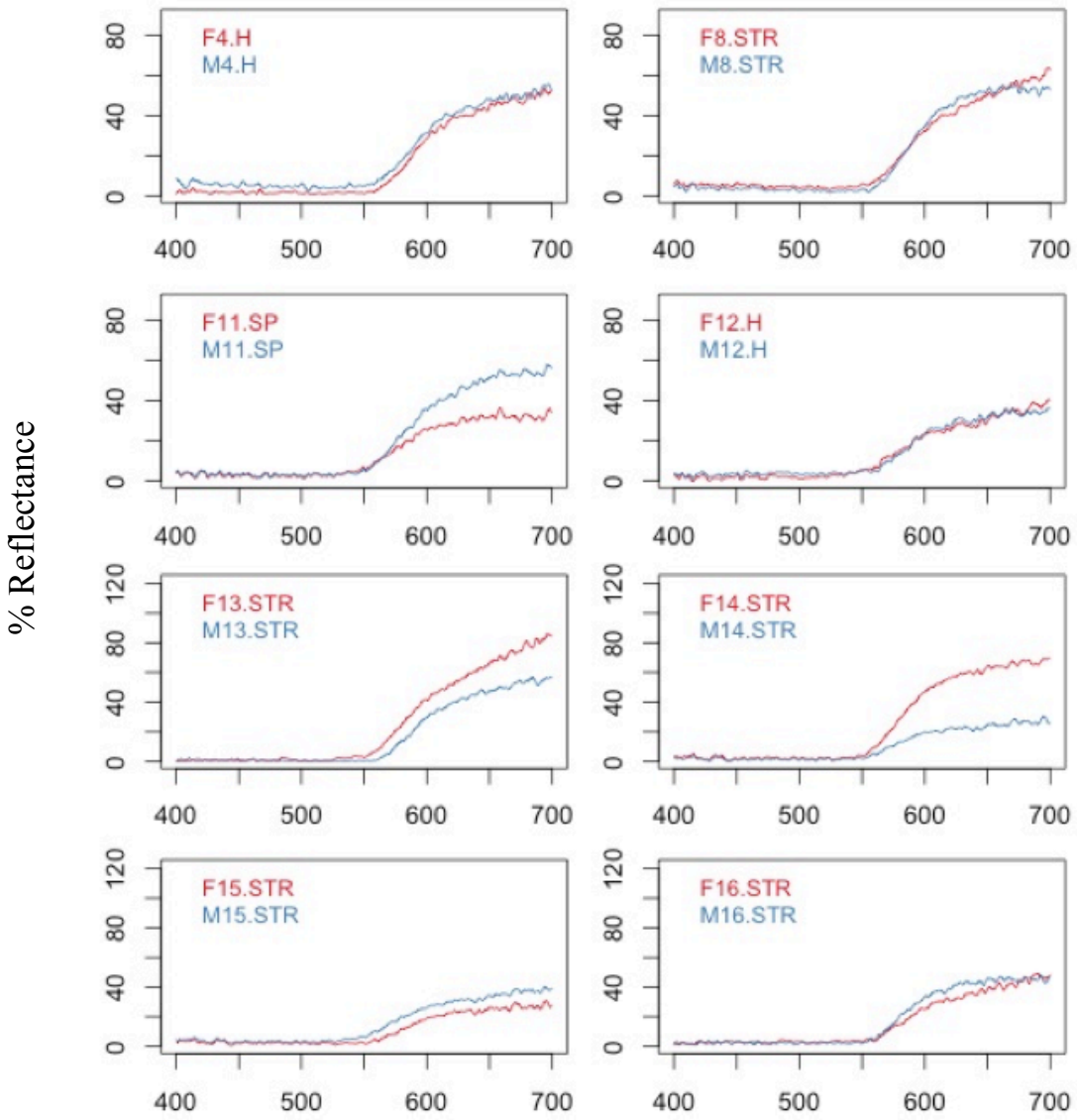
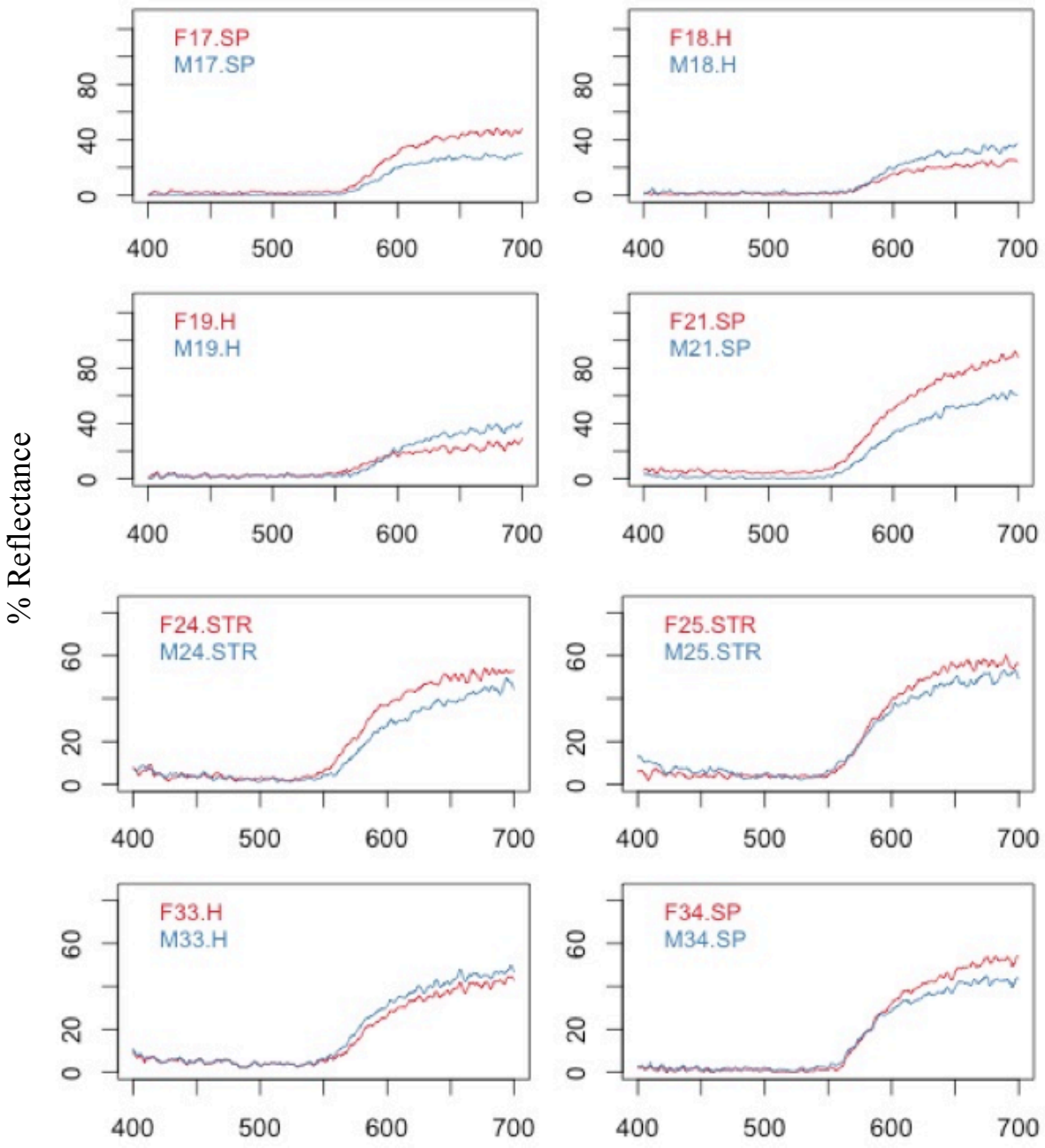
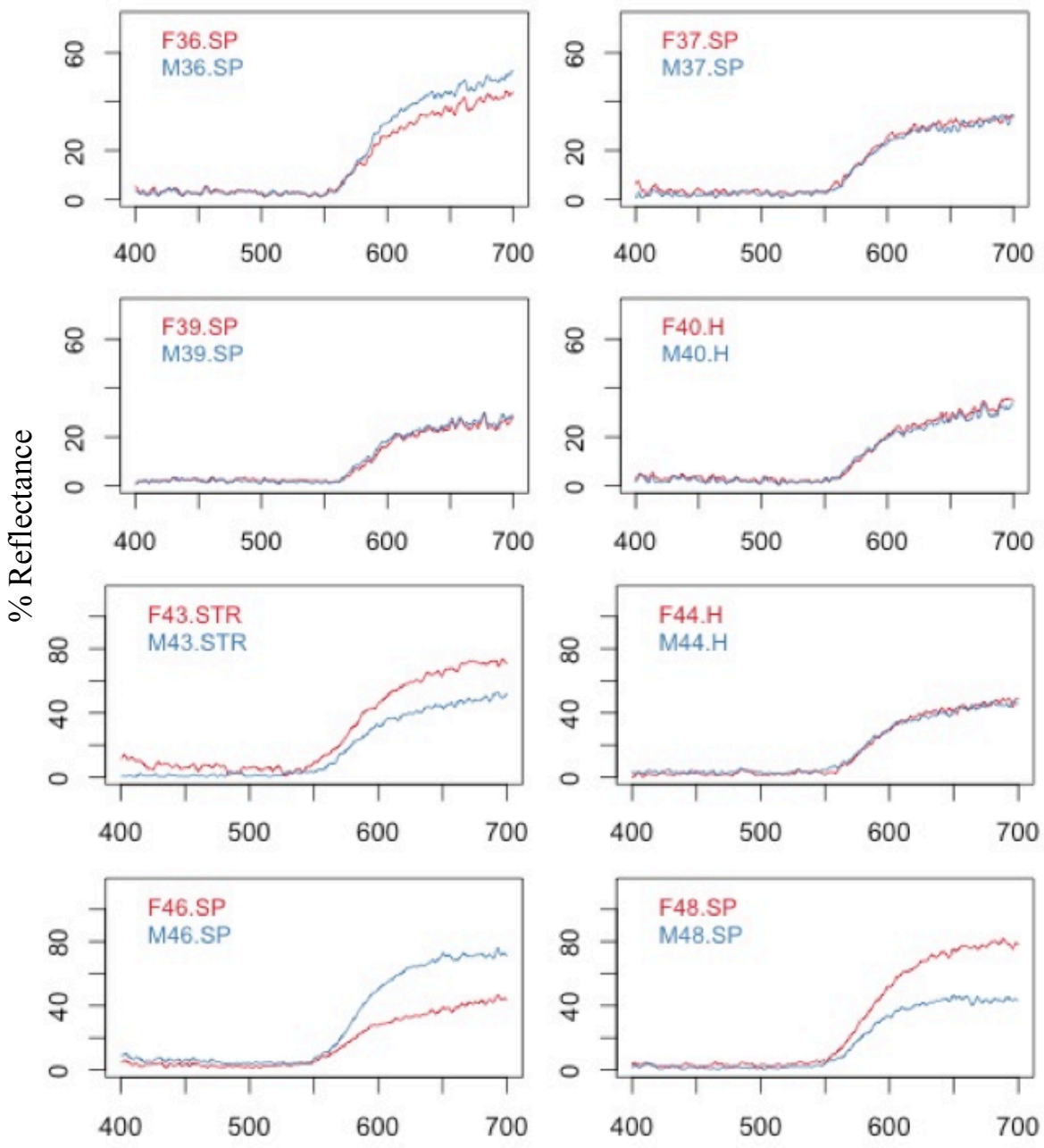


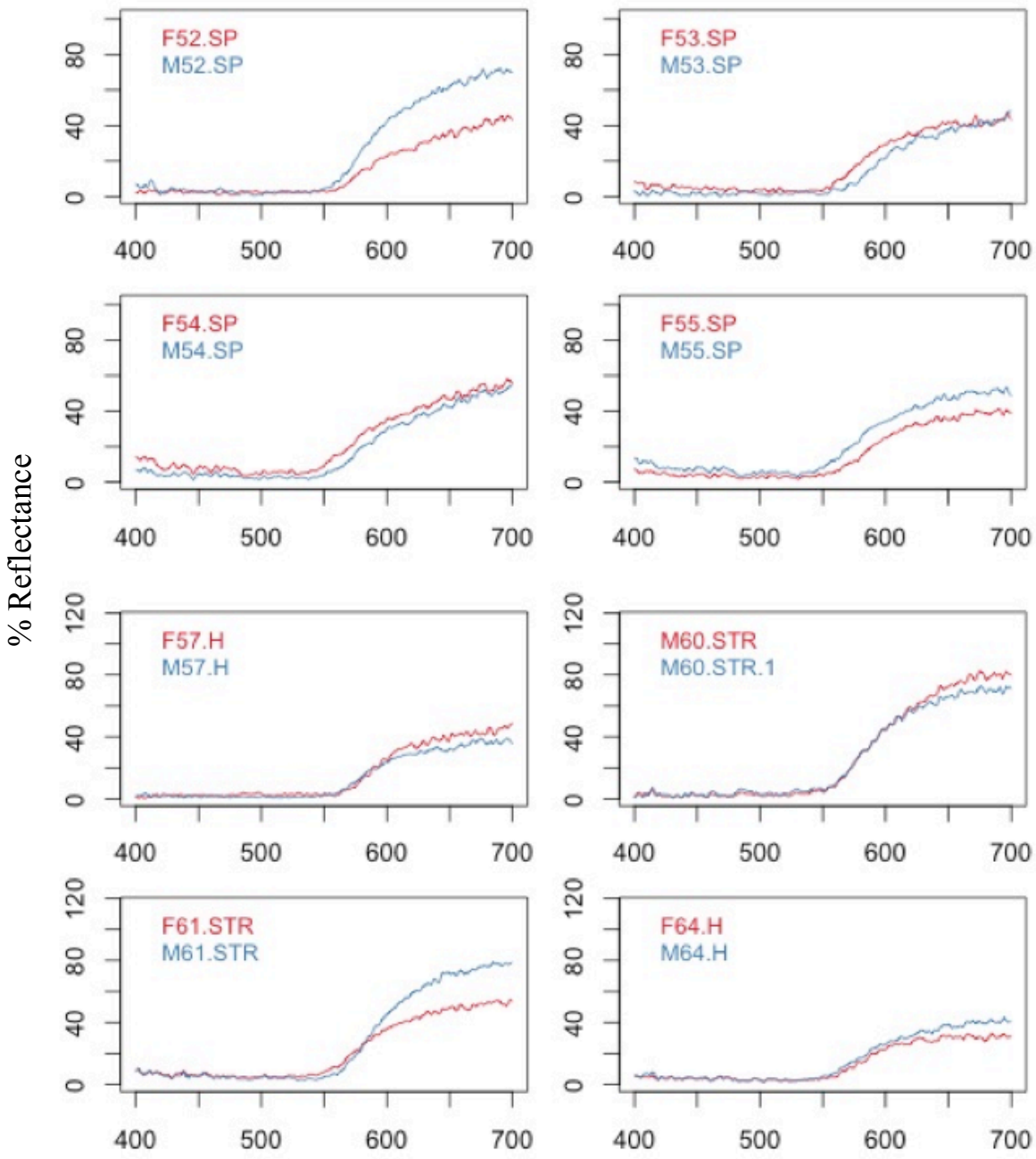
Figure 1. Standardized canonical correlates of each phenotypic trait in the correlation between "definite" mated pairs. The length of each bar represents the relative contribution of the trait to the overall correlation for both males and females, with longer bars indicating a stronger correlation.

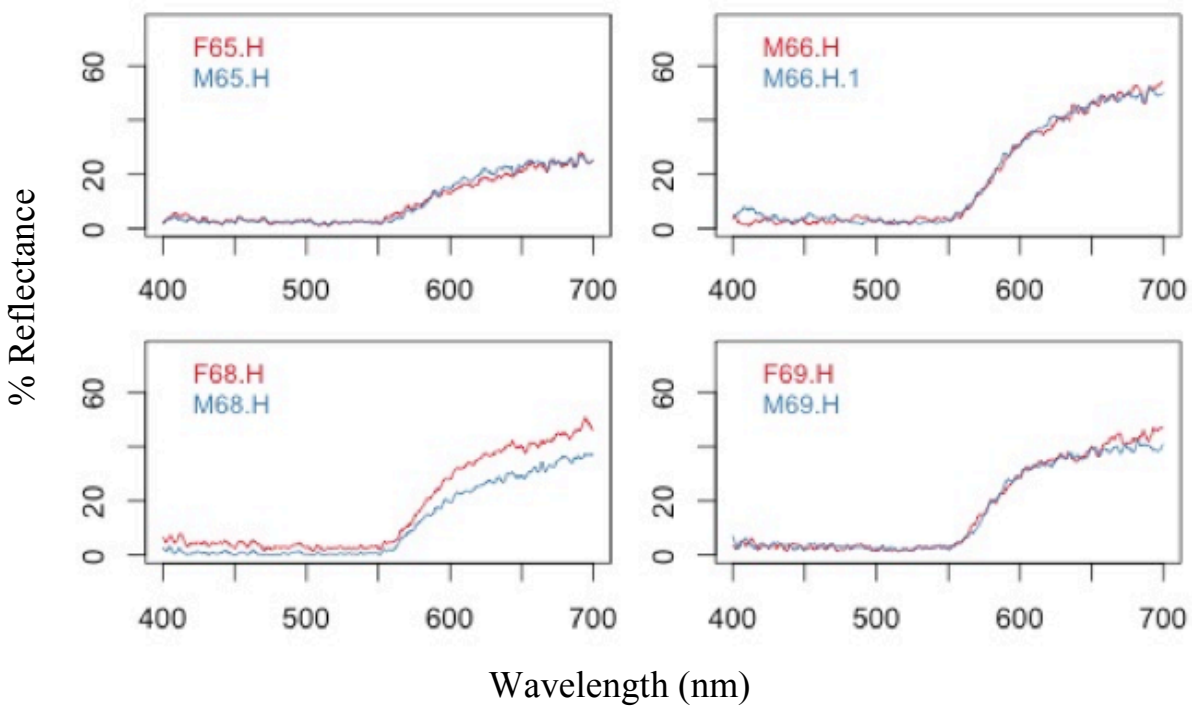
Appendix 1. Total reflectance curves for "definite" mating pairs. Red lines are female, and blue lines are males. The keys in the upper left corner indicate the sex (male (M) or Females (F)), pair identification number, and the location (Huertos (H), Successional Plot (SP), or Sendero Tres Rios (STR)) where the pair was observed.



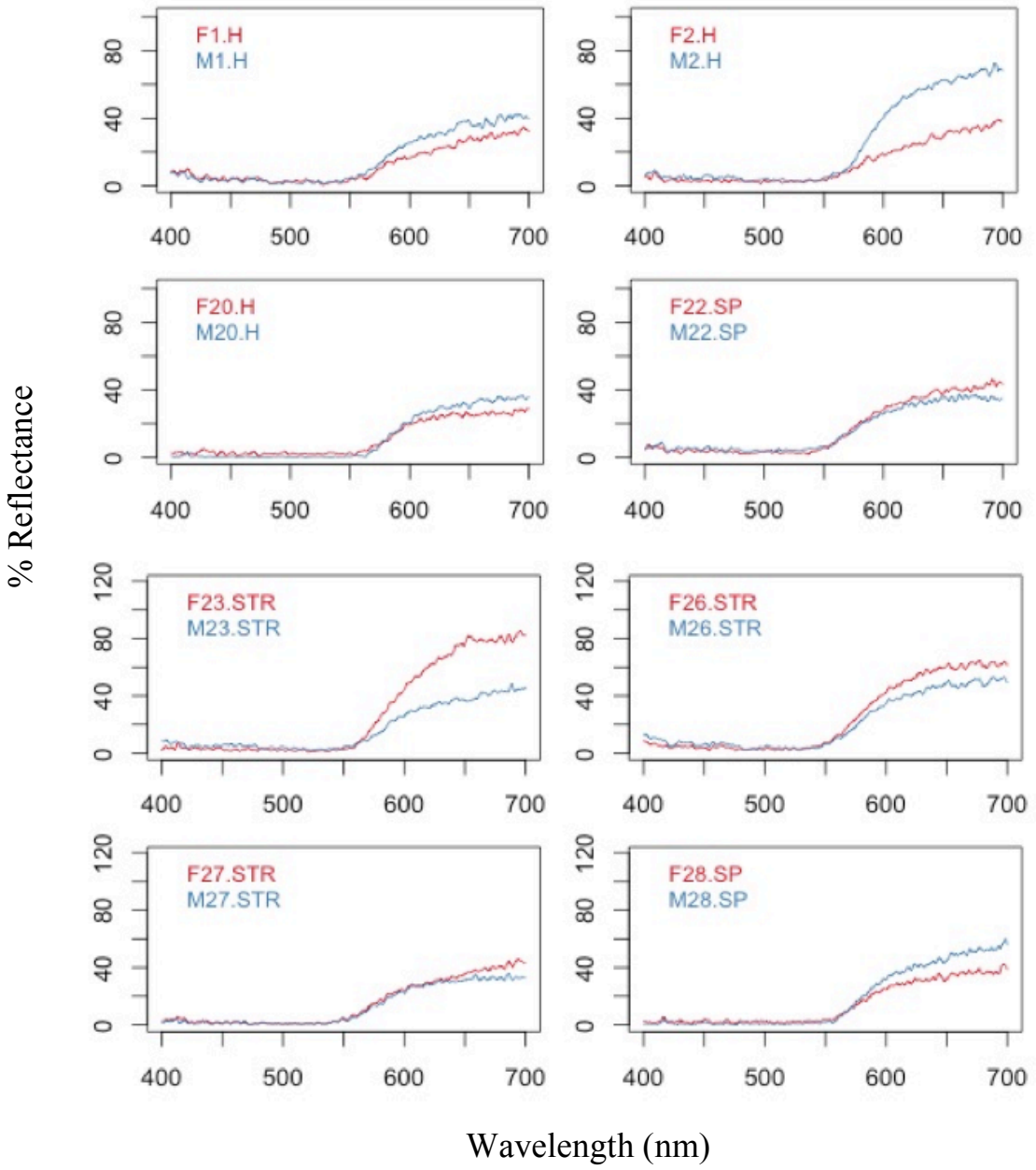

F17.SP
M17.SP
F18.H
M18.H
F19.H
M19.H
F21.SP
M21.SP
F24.STR
M24.STR
F25.STR
M25.STR
F33.H
M33.H
F34.SP
M34.SP
% Reflectance
Wavelength (nm)

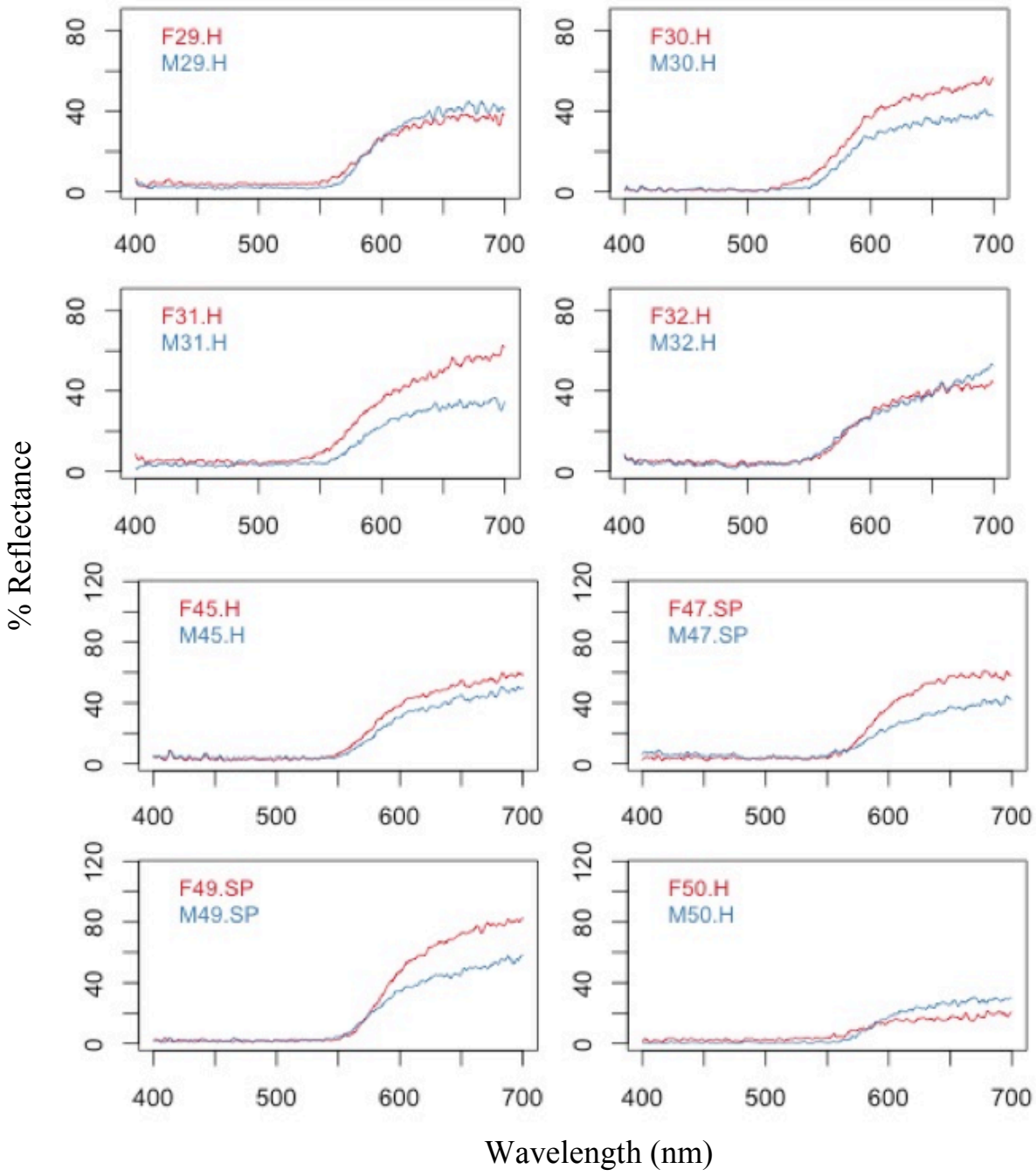

F36.SP
M36.SP
F37.SP
M37.SP
F39.SP
M39.SP
F40.H
M40.H
F43.STR
M43.STR
F44.H
M44.H
F46.SP
M46.SP
F48.SP
M48.SP
% Reflectance
Wavelength (nm)

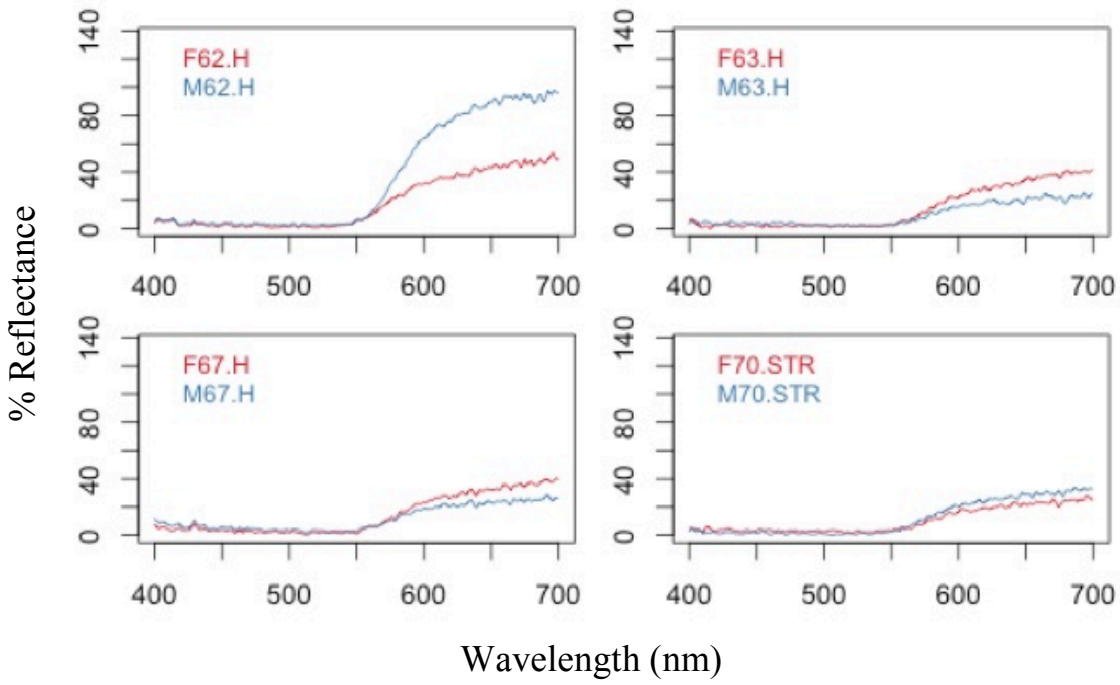

F52.SP
M52.SP
F53.SP
M53.SP
F54.SP
M54.SP
F55.SP
M55.SP
F57.H
M57.H
M60.STR
M60.STR.1
F61.STR
M61.STR
F64.H
M64.H
% Reflectance
Wavelength (nm)


% Reflectance
F65.H
M65.H
M66.H
M66.H.1
F68.H
M68.H
F69.H
M69.H
0
20
60
400
500
600
700
Wavelength (nm)

Appendix 2. Total reflectance curves for "probable" mating pairs. Red lines are female, and blue lines are males. The keys in the upper left corner indicate the sex (male (M) or Females (F)), pair identification number, and the location (Huertos (H), Successional Plot (SP), or Sendero Tres Rios (STR)) where the pair was observed.




F29.H
M29.H
F30.H
M30.H
F31.H
M31.H
F32.H
M32.H
F45.H
M45.H
F47.SP
M47.SP
F49.SP
M49.SP
F50.H
M50.H
% Reflectance
Wavelength (nm)


% Reflectance
F62.H
M62.H
F63.H
M63.H
F67.H
M67.H
F70.STR
M70.STR
0
40
80
140
400
500
600
700
Wavelength (nm)